Cellular and molecular immune markers of aging and frailty

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Appendices

Appendix A

Supplement to Chapter 2

A.1 Supplementary methods

Construction and validation of the frailty index

We constructed a frailty index to quantify health status based on previous studies (Collerton et al., 2012; K. Rockwood et al., 2011; Schoufour et al., 2017; Searle et al., 2008). Multiple health deficits were evaluated to determine their applicability in a frailty index. Deficits that could be evaluated on the basis of the latest available data from the Doetinchem Cohort Study (DCS) (round 5 (2008-2012) or the first four years of the ongoing round 6, (2013-2016)) were included if they had been used previously in an existing frailty index, if the prevalence of the deficit was at least one percent in the DCS, and if there is a known association with cognitive, physical, or psychological functioning. The final selection contained 36 deficits, including several parameters of physical functioning (e.g. handgrip strength), psychological functioning (e.g. the question 'feeling depressed last week'), cognitive functioning (e.g. cognitive memory based on the Verbal Learning Task), and the presence of a number of chronic conditions (e.g. renal dysfunction). For a full description of all deficits, see Table A.1. Health deficits were either dichotomized or trichotomized, with 0= total absence of the deficit, 0.5= partial presence of the deficit, and 1= full presence of the deficit. For instance, the deficit 'limitations in walking 100 meters' was obtained from a self-reported questionnaire and was divided in no limitation (0), some limitation (0.5), and severe limitation (1). The frailty index is calculated by dividing the number deficits in an individual by the total number of deficits investigated (n=36).

We analyzed the frailty index distribution per sex (expected to be right-skewed and higher in women(Mitnitski et al., 2001; Searle et al., 2008)) and associations between frailty and all-cause mortality to further validate it. From all Doetinchem cohort participants who gave written consent, we obtained participants' vital status information until Jan 1, 2018 from municipal personal records. Within each of the chosen 2.5-year age intervals, the frailty index of the deceased was compared with that of the survivors. To investigate whether frailty was

associated with all-cause mortality independently of age, the asymptotic Wilcoxon-Mann-Whitney test was used to estimate the overall association, combining the data from all age categories.

Within the Doetinchem Cohort Study (DCS), women had a higher frailty score than men ($p<0.001$) and for both men and women the distribution of the frailty index was right-skewed (Figure A.1a), which is consistent with previously constructed frailty indexes (Collerton et al., 2012; Mitnitski et al., 2001; Schoufour et al., 2017). Furthermore, frailty was strongly positively associated with age (Figure A.1b and A.1c) but inversely associated with educational level and smoking (all $p <0.001$). In the interval from January 2012 to January 2018, 223 out of 4311 participants died. The frailty index of the deceased individuals was consistently higher than the frailty index of the survivors in all but the lowest (<45 years) age interval (Figure A.1c). Overall, our results showed that a higher frailty index is strongly associated with all-cause mortality, also independent of age ($p<0.001$).

A.2 Supplementary figures and tables

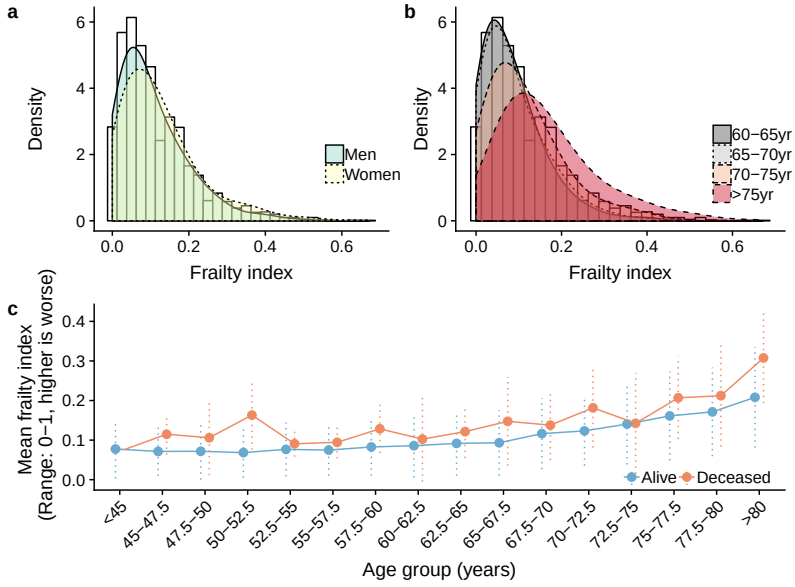

Figure A.1: Frailty index characteristics within the Doetinchem cohort. (a) Frailty index distribution per sex. (b) Frailty index density distribution per (5-year) age group. (c) Association of mortality and frailty index within the Doetinchem cohort. Colored dots show the mean frailty index grouped by (2.5-year) age category and vital status. Vital status is defined as either surviving the time period from January 2012 to January 2018 (Alive, n=4311), or dying within that time frame (Deceased, n=223).

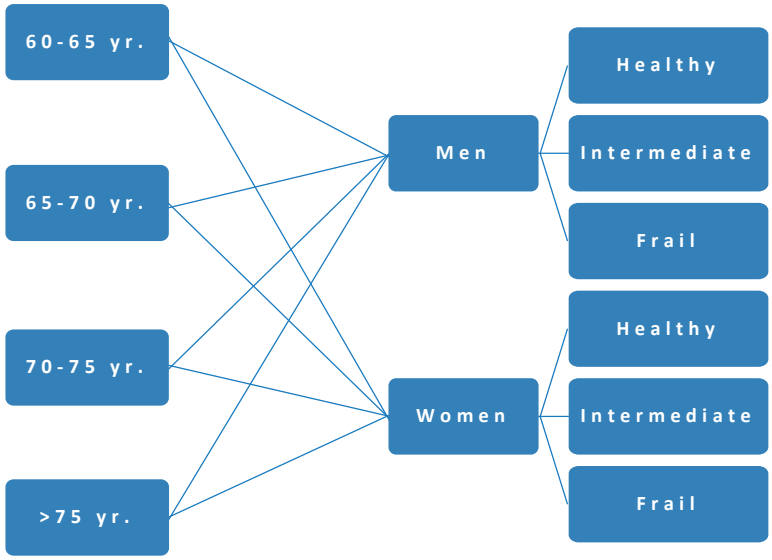

Figure A.2: Stratification scheme for sampling of individuals for DCS subcohort selection. Frail: 15% highest frailty index per age category and sex in the DCS. Healthy: 15% lowest frailty index per age category and sex in the DCS. Intermediate: participants not belonging to frail or healthy category.

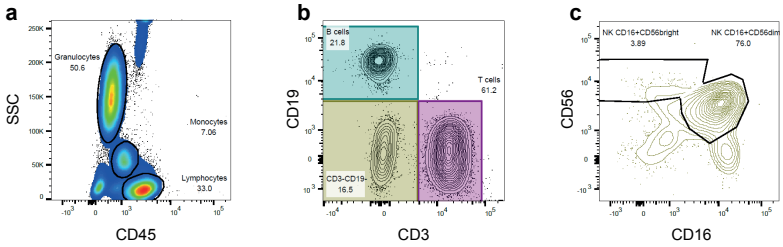

Figure A.3: Flow cytometry gating strategy to determine (a) lymphocytes, granulocytes, monocytes, (b) T cells, B cells, and (c) NK cells. SSC = Side scatter.

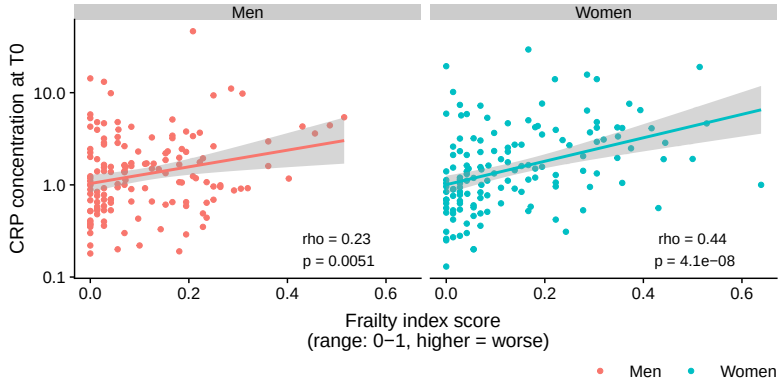

Figure A.4: The association between CRP concentrations and the frailty index score. The CRP measurements at the last time point (T0, 2008-2013) were plotted against the frailty index score for men and women separately. rho = spearman's rank correlation coefficient.

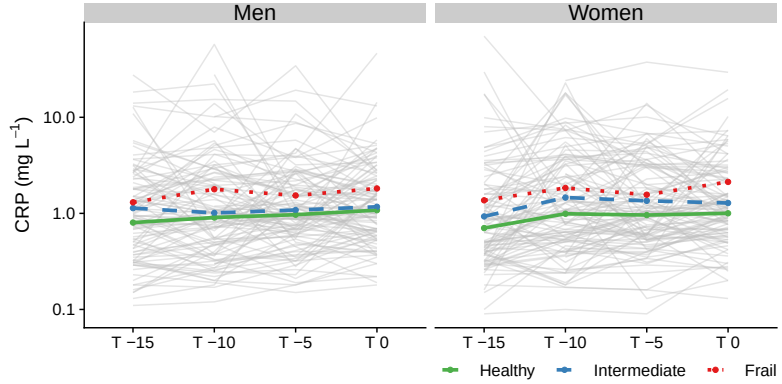

Figure A.5: CRP trajectories over a time period of approximately 15 years per frailty group, after excluding people with cardiovascular disease (CVD) and people with chronic joint inflammation. The x-axis displays the time in years according to the reference time point (`T0`). This `T0` is the moment of the most recent blood withdrawal for CRP concentration measurement. For 58% of the participants (n=106), the frailty score was assessed at `T0`. For the others (n=183), it was assessed more recently (5 years after `T0`). The colored lines show the geometric mean values per measurement time point, stratified by sex and frailty group. Significant differences between frailty and CRP levels are presented separately in Table 2.

Table A.1: Frailty index components

No	Frailty index component	Description	Value: 0	Value: 0.5	Value: 1
1	RR	High (systolic) blood pressure	$RR < 160$		$RR \geq 160$
2	Cardiologic Disease	One or more of the following conditions: myocardial infarction, bypass-surgery, balloon dilatation, cardiac catheterization, pacemaker implantation, large blood vessel surgery, hospitalization due to cardiac failure	No		One or more prevalent
3	Diabetes	Prevalence of Diabetes	No		Yes
4	Hearing	Inability to maintain a conversation in a group of 3 or more people due to hearing impairment (with hearing aid if needed)	Yes or with some effort		No or with great effort
5	Malignancy	(History of) any form of malignancy	No		Yes
6	Joint Inflammation	Chronic joint inflammation in the past year	No		Yes
7	Osteoporosis	Osteoporosis diagnosed by a medical doctor, in the past year,	No		Yes
8	Lower Back Pain	Severe lower back complaints in the past year (including lumbar herniated nucleus pulposus)	No		Yes
9	CVA	(History of) stroke	No		Yes
10	Migraine	Migraine prevalence in the past year	No		Yes
11	Neurologic Disease	One or more of the following neurological diseases, diagnosed by a medical doctor: m. Parkinson, Multiple Sclerosis, epilepsy	No		Yes

Table A.1: Frailty index components *(continued)*

No	Frailty index component	Description	Value: 0	Value: 0.5	Value: 1
12	Asthma	Asthma diagnosed by a doctor and one or more asthma attacks in the past year	No		Yes
13	Spirometry Ratio	Poor lung function quantified by spirometry measurements: first second of forced expiration divided by the forced vital capacity (FEV1/FVC)	$FEV1/FVC > 0.70$		$FEV1/FVC \leq 0.70$
14	Digestive Tract	Severe bowel disorders in the past year, diagnosed by a medical doctor	No		Yes
15	Vertigo	Vertigo with falling the past 12 months	No vertigo	Some vertigo	Severe vertigo
16	BMI	Over- or underweight	$18.5 < BMI \leq 30$		$BMI \leq 18.5 or BMI > 30$
17	Pain	Limited in daily activities due to pain	No limitation	Some limitation	Severe limitation
18	Incontinence	Unintentional urine incontinence past 12 months	No		Yes
19	Ankle Brachial Index	Poor ankle brachial index (ABI, the ratio of the systolic blood pressure in the ankle to the blood pressure in the arm)	$ABI > 0.9$		$ABI \leq 0.9$
20	Health Perception	(Subjective) perception of poor health	No or some impairment		Severe impairment

Table A.1: Frailty index components *(continued)*

No	Frailty index component	Description	Value: 0	Value: 0.5	Value: 1
21	Eyesight	Bad eyesight perception, facial recognition within 4 meters (with glasses/ contact lenses if needed)	No		Yes
22	Renal Function	Poor renal function (estimated Glomular Filtration Rate (eGFR), calculated with plasma creatinine concentrations) (Inker et al., 2012)	$eGFR \geq 60$		$eGFR < 60$
23	Cognitive Speed	Poor cognitive speed. Z-scores corrected for measurements per person and for education level. Scores derived from the Stroop Color-Word Test and the Letter-Digit Substitution Test, as described previously (Nooyens et al., 2011)	Not beloging to 10% participants with lowest z-score within the Doetinchem cohort		Belonging to 10% participants with lowest z-score within the Doetinchem cohort
24	Cognitive Memory	Poor cognitive memory. Z-scores corrected for measurements per person and for education level. Scores derived from the Verbal Learning Test, as described previously (Nooyens et al., 2011)	Not beloging to 10% participants with lowest z-score within the Doetinchem cohort		Belonging to 10% participants with lowest z-score within the Doetinchem cohort

Table A.1: Frailty index components *(continued)*

No	Frailty index component	Description	Value: 0	Value: 0.5	Value: 1
25	Cognitive Flexibility	Poor cognitive flexibility. Z-scores corrected for measurements per person and education level. Scores derived from the Stroop Color-Word Test, as described previously (Nooyens et al., 2011)	Not beloging to 10% participants with lowest z-score within the Doetinchem cohort		Belonging to 10% participants with lowest z-score within the Doetinchem cohort
26	Physical Inactive	Not meeting the Dutch healthy exercise norm.(Kemper, 2000). In addition: belonging to the 25th lowest percentile of walking activity and the 10th percentile lowest low/medium/high intensive activities in the Doetinchem cohort	No		Yes
27	ADL	Limited in washing and dressing due to health	No limitation	Some limitation	Severe limitation
28	Household	Limited in daily activities (cooking, cleaning) due to health	No limitatlion	Some limitation	Severe limitation
29	Walking	Limited in in walking 100 meters	No limitatlion	Some limitation	Severe limitation

Table A.1: Frailty index components *(continued)*

No	Frailty index component	Description	Value: 0	Value: 0.5	Value: 1
30	Lifting	Limited in lifting or carrying groceries due to health	No limitatlion	Some limitation	Severe limitation
31	Walking Stairs	Limited in climbing stairs	No limitatlion	Some limitation	Severe limitation
32	Grip Strength	Poor grip strength (cutoff points as described previously) (Fried et al., 2001)			
		$Men, BMI \leq 24$	> 29		≤ 29
		$Men, 24 < BMI \leq 26$	> 30		≤ 30
		$Men, 26 < BMI \leq 28$	> 30		≤ 30
		$Men, BMI > 28$	> 32		≤ 32
		$Women, BMI \leq 24$	> 17		≤ 17
		$Women, 23 < BMI \leq 26$	> 17.3		≤ 17.3
		$Women, 26 < BMI \leq 29$	> 18		≤ 18
		$Women, BMI > 29$	> 21		≤ 21
33	Depressed	Feeling depressed the past week	No limitatlion	Some limitation	Severe limitation
34	Happiness	Feeling unhappy the past 4 weeks	No limitatlion	Some limitation	Severe limitation

Table A.1: Frailty index components *(continued)*

No	Frailty index component	Description	Value: 0	Value: 0.5	Value: 1
35	Mental Effort	Feeling as if every activity costs effort during the past week	No limitatlion	Some limitation	Severe limitation
36	Getting Going	Not being able to get going the past week	No limitatlion	Some limitation	Severe limitation

Table A.2: Linear mixed effects regression model of CRP concentration (mg L^{-1}) for both sexes, after excluding people with CVD or joint inflammation

	log(CRP) for men			log(CRP) for women		
Predictors	Estimates	CI	p	Estimates	CI	p
(Intercept)	-0.27	-1.51 – 0.97	0.675	-0.63	-1.87 – 0.61	0.324
Frailty index	2.14	0.31 – 3.98	0.024	2.28	0.71 – 3.85	0.005
Age at baseline	0.00	-0.02 – 0.03	0.851	0.01	-0.02 – 0.03	0.531
Years after baseline age	0.01	-0.00 – 0.03	0.106	0.02	0.01 – 0.03	0.009
Observations		413			420	
Marginal R2 / Conditional R2		0.035/0.644			0.062/0.570	

Note:
CRP concentrations were log transformed before analysis. Four CRP concentration measurements per person were used, with approximately 5 years between measurements. Frailty category: the 'healthy' category was taken as the reference. Baseline age: The age at which the first CRP measurement took place. Time: time difference in years with the first baseline CRP measurement. CI= 95% confidence interval. P-values <0.05 are displayed in bold and were considered statistically significant.

Table A.3: CRP concentrations (mg L^{-1}) dependent on age and leukocyte numbers

	Men (n=145)			Women (n=144)		
Predictors	Estimates	CI	p	Estimates	CI	p
(Intercept)	-0.63	-1.61 – 0.35	0.21	-0.58	-1.79 – 0.64	0.354
Granulocytes	0.28	0.14 – 0.41	<0.001	0.40	0.21 – 0.59	<0.001
Monocytes	0.00	-0.12 – 0.12	0.954	-0.13	-0.40 – 0.14	0.33
T cells	0.10	-0.06 – 0.27	0.229	0.01	-0.16 – 0.18	0.888
B cells	-0.09	-0.29 – 0.12	0.415	0.05	-0.09 – 0.19	0.484
NK cells	0.03	-0.11 – 0.17	0.659	-0.06	-0.21 – 0.09	0.405
Age at baseline	0.01	-0.00 – 0.03	0.141	0.01	-0.01 – 0.04	0.219
Time (years after baseline age)	0.01	-0.00 – 0.02	0.09	0.02	0.01 – 0.03	0.006
Observations		533			546	
Marginal R2 / Conditional R2		0.096 / 0.607			0.105 / 0.603	

Note:
CRP concentrations were log transformed before analysis. Four CRP concentration measurements per person were used, with approximately 5 years between measurements. Cell numbers were transformed to z-scores for easier comparison. Age at baseline: age at first CRP measurement. P-values <0.05 are displayed in bold and were considered statistically significant.

Appendix B

Supplement to Chapter 3

B.1 Supplementary figures and tables

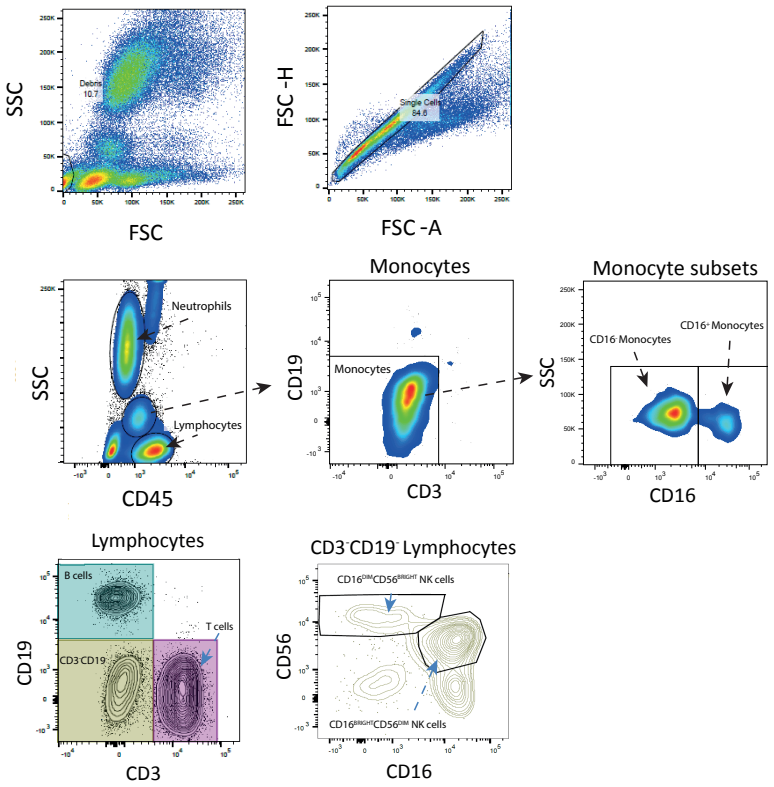

Figure B.1: General gating strategy showing gating of single cells by FSC-A versus FSC-H, leukocyte subsets by SSC and CD45, monocyte subpopulations by CD16, T and B-cells by CD3 and CD19, and NK cells by CD16 and CD56.

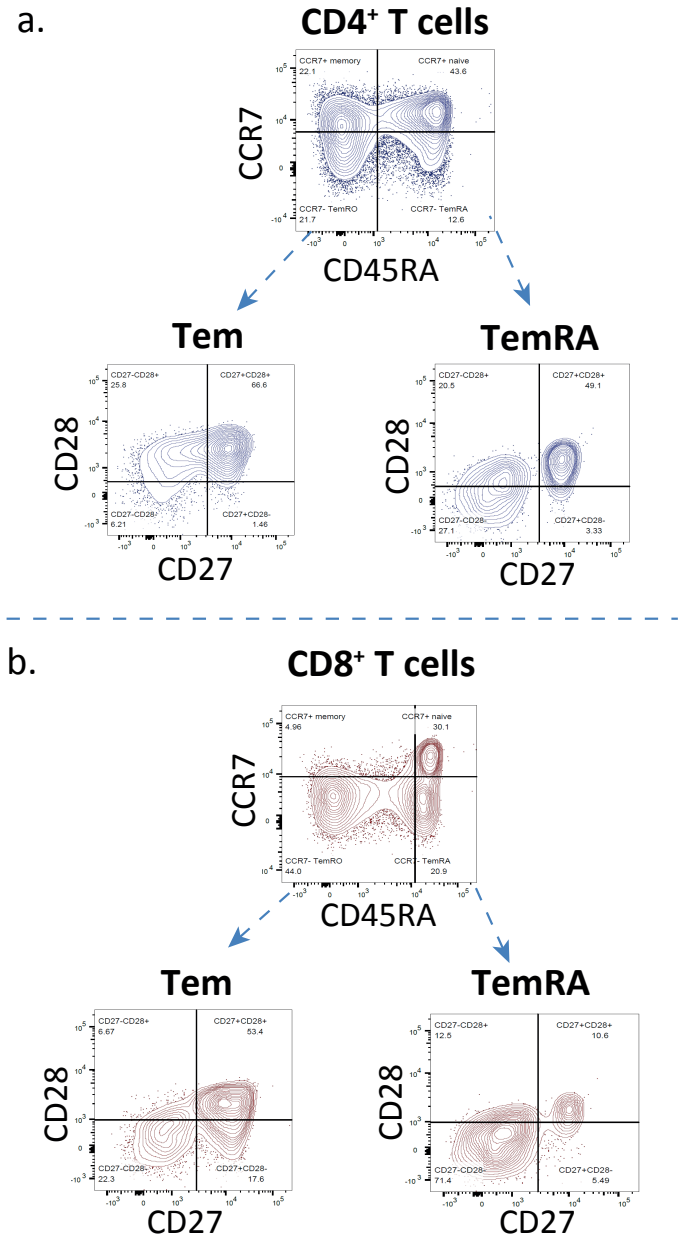

Figure B.2: Gating strategy for (a) memory $CD4^+$ T cells and (b) memory $CD8^+$ T cells. Tem: effector memory ($CD4^+$ or $CD8^+$) T cells. TemRA: effector memory T cells re-expressing CD45RA.

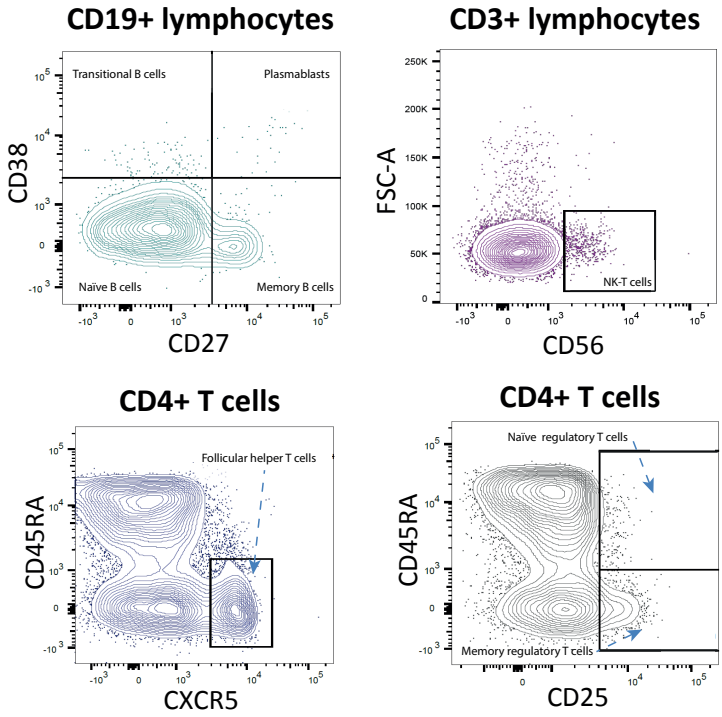

Figure B.3: Gating strategy for (a) B cell subsets and (b) follicular helper T cells and regulatory T cells.

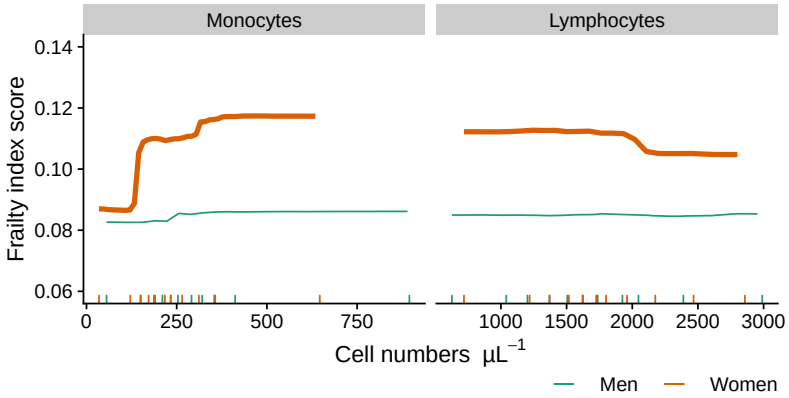

Figure B.4: Partial dependence plots showing how frailty 'depends' on total monocyte numbers and total lymphocyte numbers in men (n=140) and women (n=137). Participants with missing frailty index score data (n=12) were excluded from analysis. The short vertical segments on the horizontal axis represent the deciles of the cell numbers in the data. Range of the figures is restricted to the part containing most of the data.

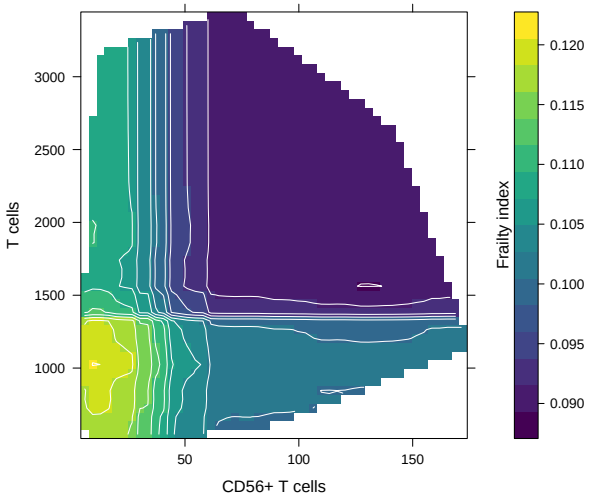

Figure B.5: Partial dependence plot with the possible joint relationship of total T cell numbers and $CD56^+$ T cell numbers on frailty. To avoid extrapolation, the plot is restricted to the area of which data is available.

Table B.1: Definition of cell phenotypes by expression of cell surface markers

Cell phenotype	Definition
T cells	
T cells	$CD45^{+}CD3^{+}$
T Follicular helper	$CD3^{+}CD4^{+}CD45RA^{-}CXCR5^{+}$
$CD56^{+}$ T cells	$CD45^{+}CD3^{+}CD56^{+}$
CD4/CD8 ratio	CD4 T cells/CD8 T cells
CD4 T cells	$CD45^{+}CD3^{+}CD4^{+}$
CD4 Naive	$CD3^{+}CD4^{+}CD45RA^{+}CCR7^{+}$
CD4 CM	$CD3^{+}CD4^{+}CD45RA^{-}CCR7^{+}$
CD4 TemRA	$CD3^{+}CD4^{+}CD45RA^{+}CCR7^{-}$
CD4 TemRA Early	$CD3^{+}CD4^{+}CD45RA^{+}CCR7^{-}CD27^{+}CD28^{+}$
CD4 TemRA Late	$CD3^{+}CD4^{+}CD45RA^{+}CCR7^{-}CD27^{-}CD28^{-}$
CD4 Tem	$CD3^{+}CD4^{+}CD45RA^{-}CCR7^{-}$
CD4 Tem Early	$CD3^{+}CD4^{+}CD45RA^{-}CCR7^{-}CD27^{+}CD28^{+}$
CD4 Tem Late	$CD3^{+}CD4^{+}CD45RA^{-}CCR7^{-}CD27^{-}CD28^{-}$
Regulatory T cells	$CD3^{+}CD4^{+}CD25^{\mathrm{BRIGHT}}$
Treg naive	$CD3^{+}CD4^{+}CD25^{\mathrm{BRIGHT}}CD45RA^{+}$
Treg memory	$CD3^{+}CD4^{+}CD25^{\mathrm{BRIGHT}}CD45RA^{-}$
CD8 T cells	$CD45^{+}CD3^{+}CD8^{+}$
CD8 Naive	$CD3^{+}CD8^{+}CD45RA^{+}CCR7^{+}$
CD8 CM	$CD3^{+}CD8^{+}CD45RA^{-}CCR7^{+}$
CD8 TemRA	$CD3^{+}CD8^{+}CD45RA^{+}CCR7^{-}$
CD8 TemRA Early	$CD3^{+}CD8^{+}CD45RA^{+}CCR7^{-}CD27^{+}CD28^{+}$
CD8 TemRA Late	$CD3^{+}CD8^{+}CD45RA^{+}CCR7^{-}CD27^{-}CD28^{-}$
CD8 Tem	$CD3^{+}CD8^{+}CD45RA^{-}CCR7^{-}$
CD8 Tem Early	$CD3^{+}CD8^{+}CD45RA^{-}CCR7^{-}CD27^{+}CD28^{+}$
CD8 Tem Late	$CD3^{+}CD8^{+}CD45RA^{-}CCR7^{-}CD27^{-}CD28^{-}$
Neutrophils	
Neutrophils	$SSC^{\mathrm{BRIGHT}}CD45^{\mathrm{DIM}}$
Neutrophils, CD16 expr.	$SSC^{\mathrm{BRIGHT}}CD45^{\mathrm{DIM}};CD16^{\mathrm{expression}}$
Monocytes	
Monocytes	$SSC^{\mathrm{DIM}}CD45^{+}CD3^{-}CD19^{-}$
$CD16^{-}$ monocytes	$SSC^{\mathrm{DIM}}CD45^{+}CD16^{-}$
$CD16^{-}$ mon. CD38 expr.	$SSC^{\mathrm{DIM}}CD45^{+}CD16^{-};CD38^{\mathrm{expression}}$
$CD16^{-}$ mon. HLADR expr.	$SSC^{\mathrm{DIM}}CD45^{+}CD16^{-};HLADR^{\mathrm{expression}}$
$CD16^{+}$ monocytes	$SSC^{\mathrm{DIM}}CD45^{+}CD16^{+}$
$CD16^{+}$ mon. CD38 expr.	$SSC^{\mathrm{DIM}}CD45^{+}CD16^{+};CD38^{\mathrm{expression}}$
$CD16^{+}$ mon. HLADR expr.	$SSC^{\mathrm{DIM}}CD45^{+}CD16^{+};HLADR^{\mathrm{expression}}$
B cells	
B cells	$CD45^{+}CD19^{+}$
Plasmablasts	$CD45^{+}CD19^{+}CD38^{\mathrm{BRIGHT}}CD27^{\mathrm{BRIGHT}}$
Transitional B cells	$CD45^{+}CD19^{+}CD38^{\mathrm{BRIGHT}}CD27^{-}$

Table B.1: Definition of cell phenotypes by expression of cell surface markers *(continued)*

Cell phenotype	Definition
Naive B cells	$CD45^{+}CD19^{+}CD38^{DIM}CD27^{-}$
Memory B cells	$CD45^{+}CD19^{+}CD38^{DIM}CD27^{+}$
NK cells	
NK cells	Either NK $CD56^{BRIGHT}$ or NK $CD56^{DIM}$
NK $CD56^{BRIGHT}$	$CD45^{+}CD3^{-}CD19^{-}CD16^{DIM}CD56^{BRIGHT}$
NK $CD56^{DIM}$	$CD45^{+}CD3^{-}CD19^{-}CD16^{BRIGHT}CD56^{DIM}$

Table B.2: Leukocyte numbers per sex and CMV serostatus

	Men			Women		
	CMV- (n=69)	CMV+ (n=76)	P value[1]	CMV- (n=53)	CMV+ (n=91)	P value[1]
T cells						
T cells	1002.6 (428.9)	1202.5 (463.4)	<0.001*	1144.7 (436.2)	1272.4 (474.9)	0.026*
T Follicular helper	49.5 (28.8)	61.8 (34.1)	0.04*	67.5 (37.6)	66.7 (40.9)	0.374
$CD56^+$ T cells	21.8 (27.8)	60.9 (108.5)	<0.001*	16 (16.7)	48.5 (61.9)	<0.001*
CD4/CD8 ratio	3.6 (2.7)	2.5 (1.8)	0.002*	4.2 (3.2)	2.8 (1.7)	<0.001*
CD4 T cells	669.7 (370.9)	766.5 (391.6)	0.041*	897.3 (371.9)	892.2 (363.3)	0.847
CD4 Naive	316.3 (283.4)	243.1 (215.2)	0.292	343.7 (262)	374.6 (295.9)	0.924
CD4 CM	175.6 (90.3)	217.1 (105)	0.01*	234.1 (118)	230.9 (149.4)	0.179
CD4 TemRA	27.8 (28.8)	46.7 (45.7)	<0.001*	33.9 (50.7)	52.6 (52.4)	0.003*
CD4 TemRA Early	22.4 (26.5)	19.7 (21.2)	0.666	29.7 (44.1)	28.2 (32.2)	0.839
CD4 TemRA Late	0.1 (0.2)	5.4 (24.2)	<0.001*	0.1 (0.2)	5.9 (13.9)	<0.001*
CD4 Tem	110.6 (70.1)	153.8 (108.3)	<0.001*	155.8 (91.6)	179.9 (112.8)	0.177
CD4 Tem Early	77.7 (51.5)	86.9 (54.3)	0.166	114.7 (59.8)	106 (73.2)	0.362
CD4 Tem Late	1.1 (1.5)	15 (25.5)	<0.001*	1.1 (1.6)	8.9 (14.2)	<0.001*
Regulatory T cells	34 (18.8)	34.7 (23.8)	0.556	42.6 (22.8)	42.1 (26.5)	0.377
Treg naive	6.7 (4.1)	7.2 (3.2)	0.607	8.3 (6.4)	9.4 (5.7)	0.479
Treg memory	9.3 (5.8)	10.2 (5.2)	0.343	10.5 (5.6)	9.1 (6.4)	0.049
CD8 T cells	188.9 (198.7)	337.2 (246.1)	<0.001*	203.5 (124.1)	333.2 (159.1)	<0.001*
CD8 Naive	20 (31.5)	18 (26.4)	0.532	35.4 (41.9)	34.1 (33.8)	0.948
CD8 CM	13.7 (11.7)	17.3 (15.6)	0.01*	17.3 (14)	16.8 (15.3)	0.76
CD8 TemRA	32.3 (36.9)	83.6 (109.9)	<0.001*	27.8 (26.9)	75 (74)	<0.001*
CD8 TemRA Early	6.3 (7.8)	7.4 (8.4)	0.73	6.7 (7.5)	9.1 (9.3)	0.129
CD8 TemRA Late	10.5 (16.1)	54.1 (66.2)	<0.001*	8.6 (13)	40.4 (49.1)	<0.001*
CD8 Tem	120.1 (116.9)	227.9 (135.7)	<0.001*	117.4 (62.9)	212.4 (111.9)	<0.001*
CD8 Tem Early	64.3 (63.6)	73 (68.1)	0.409	74.7 (53.3)	88.6 (59.3)	0.538

CD8 Tem Late	11.4 (14.5)	59 (72.8)	<0.001*	6.6 (7.8)	48.9 (55.5)	<0.001*
Neutrophils						
Neutrophils	2811.5 (2204.9)	2646.3 (1309.4)	0.197	2707.9 (1478.6)	2851.3 (1573.1)	0.733
Neutrophils, CD16 expr.[2]	3671 (5564)	3971 (5900.2)	0.393	3950 (5273)	4343 (5299)	0.86
Monocytes						
Monocytes	276.5 (136.6)	246.5 (126.3)	0.057*	233.4 (144.2)	218.4 (125.2)	0.365
$CD16^-$ monocytes	273.2 (134.9)	246 (122.1)	0.064*	229.3 (143)	211.8 (125.8)	0.351
$CD16^-$ mon. CD38 expr.[2]	1200.5 (338.2)	1273 (341.5)	0.039*	1168 (193)	1216 (285)	0.401
$CD16^-$ mon. HLADR expr.[2]	9106 (6090.2)	8736 (4603)	0.78	8834 (3806)	8171 (6208)	0.907
$CD16^+$ monocytes	4 (4.7)	4 (3.9)	0.397	4 (3.8)	3.4 (4.2)	0.628
$CD16^+$ mon. CD38 expr.[2]	445.5 (192.5)	499 (199.5)	0.157	412 (139)	444 (218)	0.138
$CD16^+$ mon. HLADR expr.[2]	56851 (27956)	52916 (41323.5)	0.587	42036 (41637)	39669 (39328)	0.935
B cells						
B cells	124 (86.8)	150.4 (104.2)	0.427	167.7 (115.2)	173.9 (118.8)	0.904
Plasmablasts	1.5 (1.5)	1.4 (1.4)	0.677	1.5 (1.5)	1.4 (1.2)	0.644
Transitional B cells	3.7 (3.7)	3.8 (5.6)	0.643	4.6 (4.8)	4.2 (4.4)	0.806
Naive B cells	92.1 (72.8)	111 (75.6)	0.487	124.8 (88.9)	120.4 (78.1)	0.826
Memory B cells	24 (24.4)	28.4 (30)	0.73	32.8 (33.4)	37.1 (32.2)	0.667
NK cells						
NK cells	274.9 (217)	217.4 (191.2)	0.109	236.1 (117)	212.3 (138.8)	0.39
NK $CD56^{BRIGHT}$	10.7 (7.2)	10.4 (7.7)	0.872	11.6 (8.9)	9.9 (6.6)	0.199
NK $CD56^{DIM}$	251 (219.4)	203.1 (183.8)	0.116	220.8 (109.8)	204.6 (139.5)	0.46

Note:

All values are median (interquartile range) cell numbers per µL, unless otherwise stated

[1] P values of Kruskal-Wallis rank sum test between CMV serostatus per sex, adjusted for age

[2] Values in Median fluoresence intensity

* Selected outcomes when the False Discovery Rate is set to a maximum of 15%

Table B.3: Spearman associations between immune cell subpopulations and frailty in men (n=140)

Immune cell subset	ρ	P value	FDR*
Neutrophils	**0.25**	**0.002**	**0.077**
Transitional B cells	-0.19	0.015	0.324
Monocytes	0.17	0.027	0.389
$CD16^-$ monocytes	0.16	0.036	0.383
CD4 Naive	0.17	0.040	0.341
CD4 TemRA Late	0.13	0.078	0.562
CD4 TemRA	0.17	0.090	0.553
Regulatory T cells	0.12	0.104	0.561
$CD16^+$ monocytes	0.16	0.133	0.633
$CD16^-$ mon. HLADR expr.	-0.15	0.133	0.571
NK $CD56^{DIM}$	-0.08	0.172	0.672
CD4 TemRA Early	0.14	0.191	0.686
CD4 T cells	0.09	0.207	0.683
NK cells	-0.06	0.208	0.638
CD4/CD8 ratio	0.10	0.219	0.628
Memory B cells	-0.07	0.319	0.857
T cells	0.09	0.321	0.812
CD8 Tem Early	-0.07	0.354	0.845
Treg naive	0.08	0.387	0.877
Neutrophils, CD16 expr.	-0.10	0.399	0.858
T Follicular helper	0.07	0.439	0.898
$CD16^+$ mon. CD38 expr.	-0.05	0.440	0.860
Lymphocytes	0.04	0.574	1.073
NK $CD56^{BRIGHT}$	-0.03	0.582	1.042
CD8 Naive	0.05	0.590	1.014
CD8 CM	-0.05	0.650	1.074
CD8 T cells	0.03	0.658	1.048
B cells	-0.06	0.667	1.025
CD8 Tem Late	0.09	0.689	1.022
$CD56^+$ T cells	0.03	0.694	0.995
CD8 Tem	0.02	0.697	0.967
$CD16^-$ mon. CD38 expr.	-0.01	0.705	0.948
$CD16^+$ mon. HLADR expr.	0.07	0.728	0.949
CD4 Tem Late	0.03	0.735	0.930

Table B.3: Spearman associations between immune cell subpopulati *(continued)*

Immune cell subset	ρ	P value	FDR*
CD4 CM	-0.05	0.757	0.931
CD4 Tem Early	-0.03	0.789	0.943
CD8 TemRA Late	0.02	0.796	0.925
Plasmablasts	0.04	0.810	0.916
Naive B cells	-0.05	0.839	0.925
CD8 TemRA	0.02	0.851	0.915
CD8 TemRA Early	-0.01	0.931	0.976
Treg memory	0.03	0.967	0.990
CD4 Tem	0.00	0.994	0.994

Note:
The (Spearman) associations are ordered by p value, with the lowest p values shown at the top. Associations that were selected with a FDR lower than 15% are shown in bold.
* False Discovery Rate (estimated)

Table B.4: Spearman associations between immune cell subpopulations and frailty in women (n=137)

Immune cell subset	ρ	P value	FDR*
Neutrophils	**0.40**	**<0.001**	**0.000**
CD16$^-$ monocytes	**0.24**	**0.003**	**0.066**
Monocytes	**0.23**	**0.004**	**0.054**
CD56$^+$ T cells	**-0.20**	**0.01**	**0.109**
CD4 TemRA Late	**-0.13**	**0.015**	**0.128**
CD16$^-$ mon. CD38 expr.	-0.20	0.022	0.157
CD4 Tem Early	0.20	0.032	0.194
CD16$^+$ mon. CD38 expr.	-0.18	0.037	0.200
CD16$^-$ mon. HLADR expr.	-0.12	0.063	0.302
CD4 Tem	0.15	0.073	0.313
NK CD56$^{\text{BRIGHT}}$	-0.15	0.08	0.311
CD8 TemRA	-0.09	0.091	0.325
Transitional B cells	-0.16	0.096	0.318
CD8 TemRA Late	-0.08	0.099	0.303
NK CD56$^{\text{DIM}}$	-0.15	0.133	0.382
NK cells	-0.13	0.149	0.401
CD8 CM	0.14	0.165	0.418
CD4 Tem Late	-0.04	0.235	0.562
CD4/CD8 ratio	0.11	0.25	0.567
CD4 TemRA Early	0.09	0.274	0.589
CD8 T cells	-0.10	0.322	0.658
CD4 CM	0.07	0.34	0.665
CD4 Naive	-0.11	0.344	0.643
T Follicular helper	0.09	0.347	0.621
CD8 Naive	-0.13	0.359	0.618
Treg memory	0.07	0.374	0.618
CD8 TemRA Early	-0.02	0.422	0.672
Memory B cells	-0.09	0.428	0.658
Plasmablasts	-0.07	0.461	0.683
T cells	-0.05	0.49	0.702
CD8 Tem Early	0.12	0.519	0.720
Lymphocytes	-0.02	0.526	0.707
CD16$^+$ monocytes	0.07	0.551	0.718
B cells	-0.06	0.579	0.732

Table B.4: Spearman associations between immune cell subpopulati *(continued)*

Immune cell subset	ρ	P value	FDR*
Neutrophils, CD16 expr.	-0.01	0.73	0.897
Treg naive	-0.04	0.779	0.930
CD8 Tem	0.02	0.814	0.946
CD4 TemRA	0.00	0.913	1.033
$CD16^+$ mon. HLADR expr.	0.01	0.946	1.044
CD8 Tem Late	0.01	0.953	1.025
Regulatory T cells	0.01	0.966	1.013
CD4 T cells	0.01	0.967	0.990
Naive B cells	0.00	0.984	0.984

Note:
The (Spearman) associations are ordered by p value, with the lowest p values shown at the top. Associations that were selected with a FDR lower than 15% are shown in bold.
* False Discovery Rate (estimated)

Table B.5: Repeated sensitivity analysis in men when monocytes were restricted to be HLADR$^+$

Immune cell subset	ρ	P value	FDR*
Neutrophils	**0.25**	**0.002**	**0.061**
Transitional B cells	-0.19	0.014	0.276
Monocytes (HLADR$^+$)	0.17	0.027	0.349
CD4 Naive	0.17	0.040	0.386
CD16$^-$ monocytes (HLADR$^+$)	0.14	0.048	0.378
CD4 TemRA Late	0.13	0.078	0.508
CD4 TemRA	0.17	0.091	0.508
Regulatory T cells	0.12	0.104	0.508
CD16$^+$ monocytes (HLADR$^+$)	0.17	0.108	0.470
NK CD56$^{\text{DIM}}$	-0.08	0.172	0.671
CD4 TemRA Early	0.14	0.191	0.677
CD4 T cells	0.09	0.205	0.666
NK cells	-0.06	0.209	0.626
CD4/CD8 ratio	0.10	0.222	0.617
T cells	0.09	0.319	0.828
Memory B cells	-0.07	0.320	0.780
CD8 Tem Early	-0.07	0.354	0.812
Treg naive	0.08	0.390	0.844
Neutrophils, CD16 expr.	-0.10	0.401	0.822
T Follicular helper	0.07	0.441	0.860
Lymphocytes	0.04	0.574	1.066
NK CD56$^{\text{BRIGHT}}$	-0.03	0.580	1.029
CD8 Naive	0.05	0.589	0.999
CD8 CM	-0.05	0.651	1.058
CD8 T cells	0.03	0.657	1.026
B cells	-0.06	0.669	1.003
CD8 Tem Late	0.09	0.688	0.994
CD56$^+$ T cells	0.03	0.695	0.968
CD8 Tem	0.02	0.697	0.938
CD4 Tem Late	0.03	0.734	0.954
CD4 CM	-0.05	0.756	0.951
CD4 Tem Early	-0.03	0.789	0.962
CD8 TemRA Late	0.02	0.794	0.938
Plasmablasts	0.04	0.807	0.926

Table B.5: Repeated sensitivity analysis in men when monocytes were restricted to be HLADR$^+$ *(continued)*

Immune cell subset	ρ	P value	FDR*
Naive B cells	-0.05	0.838	0.934
CD8 TemRA	0.02	0.853	0.924
CD8 TemRA Early	-0.01	0.929	0.980
Treg memory	0.03	0.966	0.992
CD4 Tem	0.00	0.994	0.994

Note:
The (Spearman) associations are ordered by p value, with the lowest p values shown at the top. Associations that were selected with a FDR lower than 15% are shown in bold

* False Discovery Rate (estimated)

Table B.6: Repeated sensitivity analysis in women when monocytes were restricted to be HLADR$^+$

Immune cell subset	ρ	P value	FDR*
Neutrophils	**0.40**	**<0.001**	**0.000**
Monocytes (HLADR$^+$)	**0.23**	**0.004**	**0.074**
CD56$^+$ T cells	**-0.20**	**0.01**	**0.124**
CD16$^-$ monocytes (HLADR$^+$)	**0.18**	**0.014**	**0.139**
CD4 TemRA Late	**-0.13**	**0.015**	**0.120**
CD4 Tem Early	0.20	0.032	0.208
CD4 Tem	0.15	0.071	0.395
NK CD56$^{\text{BRIGHT}}$	-0.15	0.078	0.380
CD8 TemRA	-0.09	0.09	0.390
Transitional B cells	-0.16	0.096	0.375
CD8 TemRA Late	-0.08	0.099	0.350
NK CD56$^{\text{DIM}}$	-0.15	0.133	0.433
NK cells	-0.13	0.149	0.446
CD8 CM	0.14	0.167	0.466
CD4 Tem Late	-0.04	0.235	0.610
CD4/CD8 ratio	0.11	0.251	0.612
CD4 TemRA Early	0.09	0.274	0.628
CD8 T cells	-0.10	0.324	0.701
CD4 CM	0.07	0.34	0.697
T Follicular helper	0.09	0.345	0.674
CD4 Naive	-0.11	0.346	0.642
CD8 Naive	-0.13	0.359	0.637
Treg memory	0.07	0.375	0.636
CD8 TemRA Early	-0.02	0.423	0.688
Memory B cells	-0.09	0.428	0.668
CD16$^+$ monocytes (HLADR$^+$)	0.09	0.441	0.661
Plasmablasts	-0.07	0.458	0.662
T cells	-0.05	0.49	0.683
CD8 Tem Early	0.12	0.521	0.701
Lymphocytes	-0.02	0.528	0.686
B cells	-0.06	0.578	0.727
Neutrophils, CD16 expr.	-0.01	0.729	0.888
Treg naive	-0.04	0.78	0.921
CD8 Tem	0.02	0.814	0.934

Table B.6: Repeated sensitivity analysis in women when monocytes were restricted to be HLADR$^+$ *(continued)*

Immune cell subset	ρ	P value	FDR*
CD4 TemRA	0.00	0.914	1.018
CD8 Tem Late	0.01	0.953	1.032
Regulatory T cells	0.01	0.965	1.017
CD4 T cells	0.01	0.967	0.992
Naive B cells	0.00	0.985	0.985

Note:
The (Spearman) associations are ordered by p value, with the lowest p values shown at the top. Associations that were selected with a FDR lower than 15% are shown in bold
* False Discovery Rate (estimated)

Table B.7: Additional analysis in men (n=140) to test associations with frailty when percentages of subpopulations are used instead of absolute numbers

Immune cell subset	ρ	P value	FDR*
Transitional B cells, (%)	-0.22	0.011	0.373
CD4 Naive, (%)	0.22	0.018	0.302
CD4 CM, (%)	-0.24	0.023	0.260
T cells, (%)	0.16	0.047	0.395
NK cells, (%)	-0.11	0.091	0.621
CD4 TemRA Late, (%)	0.11	0.169	0.958
B cells, (%)	-0.14	0.181	0.880
NK $CD56^{BRIGHT}$, (%)	0.10	0.185	0.787
CD4 Tem, (%)	-0.10	0.186	0.702
CD4 Tem Early, (%)	-0.11	0.204	0.695
CD8 T cells, (%)	-0.11	0.209	0.644
CD4 T cells, (%)	0.12	0.213	0.603
Treg memory, (%)	-0.09	0.215	0.562
NK $CD56^{DIM}$, (%)	-0.08	0.247	0.600
CD4 TemRA, (%)	0.10	0.260	0.589
CD8 Naive, (%)	0.08	0.342	0.727
Regulatory T cells, (%)	0.05	0.425	0.849
CD8 CM, (%)	-0.03	0.464	0.877
CD8 Tem Early, (%)	-0.07	0.469	0.840
CD8 Tem Late, (%)	0.11	0.474	0.806
CD4 TemRA Early, (%)	0.06	0.573	0.927
Plasmablasts, (%)	0.04	0.582	0.900
Memory B cells, (%)	-0.01	0.670	0.990
CD8 TemRA, (%)	0.03	0.676	0.958
Naive B cells, (%)	0.01	0.705	0.958
CD8 TemRA Late, (%)	0.00	0.719	0.940
CD8 TemRA Early, (%)	0.00	0.769	0.969
T Follicular helper, (%)	-0.03	0.793	0.963
$CD16^{-}$ monocytes, (%)	0.01	0.838	0.983
CD4 Tem Late, (%)	-0.05	0.848	0.961
CD8 Tem, (%)	0.08	0.865	0.948
$CD16^{+}$ monocytes, (%)	-0.01	0.890	0.945
Treg naive, (%)	-0.02	0.934	0.962

Table B.7: Additional analysis in men (n=140) to test associations with frailty when percentages of subpopulations are used instead of absolute numbers *(continued)*

Immune cell subset	ρ	P value	FDR*
$CD56^+$ T cells, (%)	-0.03	0.992	0.992

Note:
The (Spearman) associations are ordered by p value, with the lowest p values shown at the top. Associations that were selected with a FDR lower than 15% are shown in bold
* False Discovery Rate (estimated)

Table B.8: Additional analysis in women (n=137) to test associations with frailty when percentages of subpopulations are used instead of absolute numbers

Immune cell subset	ρ	P value	FDR*
$CD56^+$ T cells, (%)	-0.18	0.009	0.304
CD4 TemRA Late, (%)	-0.17	0.009	0.156
CD8 Tem, (%)	0.20	0.021	0.236
Transitional B cells, (%)	-0.21	0.022	0.188
CD4 Tem Early, (%)	0.17	0.029	0.195
CD8 Tem Early, (%)	0.18	0.056	0.319
CD8 CM, (%)	0.14	0.062	0.301
CD8 TemRA Late, (%)	-0.09	0.065	0.275
CD4 Tem, (%)	0.16	0.066	0.251
CD8 TemRA, (%)	-0.09	0.075	0.256
Naive B cells, (%)	0.13	0.086	0.264
NK cells, (%)	-0.17	0.093	0.264
CD4 Naive, (%)	-0.12	0.133	0.347
CD4 CM, (%)	0.14	0.138	0.336
CD4 T cells, (%)	0.10	0.205	0.465
Treg memory, (%)	0.07	0.229	0.486
CD4 Tem Late, (%)	-0.05	0.232	0.465
CD4 TemRA Early, (%)	0.10	0.258	0.487
T cells, (%)	0.16	0.293	0.524
CD8 T cells, (%)	-0.09	0.294	0.499
Memory B cells, (%)	-0.08	0.334	0.540
T Follicular helper, (%)	0.10	0.350	0.540
$CD16^+$ monocytes, (%)	-0.02	0.458	0.677
Treg naive, (%)	-0.05	0.460	0.651
CD8 Tem Late, (%)	0.09	0.480	0.653
$CD16^-$ monocytes, (%)	0.07	0.547	0.716
B cells, (%)	-0.06	0.572	0.720
CD8 Naive, (%)	-0.10	0.662	0.803
Plasmablasts, (%)	-0.04	0.694	0.814
Regulatory T cells, (%)	-0.04	0.726	0.823
CD8 TemRA Early, (%)	-0.04	0.728	0.798
NK $CD56^{DIM}$, (%)	0.02	0.825	0.877
NK $CD56^{BRIGHT}$, (%)	-0.02	0.825	0.850

Table B.8: Additional analysis in women (n=137) to test associations with frailty when percentages of subpopulations are used instead of absolute numbers *(continued)*

Immune cell subset	ρ	P value	FDR*
CD4 TemRA, (%)	0.00	0.893	0.893

Note:
The (Spearman) associations are ordered by p value, with the lowest p values shown at the top. Associations that were selected with a FDR lower than 15% are shown in bold
* False Discovery Rate (estimated)

Appendix C

Supplement to Chapter 4

C.1 Supplementary methods

The initial response of the random sample of the population was 62%. Two-third* of those who agreed to be measured again were approached for the second measurement 6 years later (T1 in our study). Everybody was invited for the subsequent measurements every 5 years with exclusion of those who died, moved too far away, emigrated and those who actively withdrew from the study. Respondents can skip one or more rounds and then participate again. Per round, 2% - 3% of the participants died or moved. From T2 onward, in every round 23% of those invited did not participate with almost 50% of them mentioning that they do not want to participate again or not this time (reasons given: no time, not interested, already often medically examined) and from 50% we got no response

Description HAI components

Systolic blood pressure

SBP was measured twice while the participant was in an upright sitting position. The two measurements were averaged. The categorization for the HAI was as follows: 2 = below 126

mmHg; 1 = 126-142 mmHg; 0 = above or equal to 143 mmHg. This is a similar to classification applied in previous studies (5, 6, 8). Subjects who reported a physician diagnosis of hypertension, or who were taking medication for hypertension, were classified in the most unhealthy category (score = 0).

Random blood glucose

Random blood glucose (RBG) was determined in a peripheral blood plasma sample. Diabetes is diagnosed when the random glucose concentration is 11.1 mmol L^{-1} or higher. Hence, all values equal or above 11.1 mmol L^{-1} were assigned a score of 0. The International Diabetes Federation recommends additional screening for individuals with values between 5.6 – 11.1 mmol L^{-1} (17). Moreover, Bowen, Xuan (18) showed that a RBG $\geq$ 5.6 mmol L^{-1} was more strongly associated with undiagnosed diabetes than any single risk factor and remained strongly associated with undiagnosed diabetes after adjustment for traditional diabetes risk factors. Hence, values between 5.6 – 11.1 mmol L^{-1} were assigned a score of 1. Consequently, all values below 5.6 mmol L^{-1} received a score of 2. Participants who reported a physician diagnosis of diabetes or who were using medication for diabetes were coded in the highest RBG group (=0).

Creatinine

Several biochemical markers were measured in all the available samples of the follow up rounds. We used plasma creatinine levels, which are associated with renal function (19). The cut-off points for creatinine were sex-specific and replicated from previous studies (6, 8). The cut-off points for women were the following: 2 = below 70.7 mmol L^{-1}; 1 = 70.7 – 88.4 mmol L^{-1}; 0 = above or equal to 88.4 mmol L^{-1}. For men the cut-off points were the following: 2 = below 97.2 mmol L^{-1}; 1 = 97.2 – 114.9 mmol L^{-1}; 0 = above or equal to 114.9 mmol L^{-1}.

Forced vital capacity (FVC)

Pulmonary function measurements were performed by trained paramedics using a heated pneumotachometer. Participant's expiratory pulmonary volume was measured in a sitting position while wearing a nose clip. At least three technically acceptable attempts for measuring FVC had to be achieved, of which two had to be reproducible according to ERS criteria (20). For comparability to other studies, the FVC was used as an indicator for lung function. Cut-off points were sex-specific, with the following values for FVC in men: 2 = above or equal to 3.84 L; 1 = 3.19-3.84 L; and 0 = below 3.19 L. For FVC in women, scores were the following: 2 = above or equal to 2.61 L; 1 = 2.14-2.61 L; 0 = below 2.14 L.

Cognitive function

Cognition was only measured in the study population aged 45 years and older. Cognitive function was measured by four neuropsychological tests: the 15 Words Verbal Learning Test (immediate and delayed recall), the Stroop Color-Word-Test, the Word Fluency Test and the Letter Digit Substitution Test. Nooyens, Bueno-de-Mesquita (21) have described these cognitive tests in more detail. From the separate test scores a summary score of global cognitive functioning was calculated. To capture the decline rate over time, the global cognitive functioning score was transformed into a z-score which was derived from the values of all the rounds together. Participants scoring below the 10th percentile on the global cognitive functioning z-score in T4 were considered cognitively frail, and assigned a score of 0 assigned for cognition. This cut-off point is consistent with the definition for cognitive frailty used in the DCS previously (22, 23). The z-score that corresponded to the 10th percentile in T4 (z-score = -1.05) was applied as the cut-off point for cognitive frailty in the other rounds of the DCS as well. Participants with a z-score between -1.05 – 0 were assigned a score of '1,' while participants with a z-score above 0

received a score of '2.' As cognitive tests were only performed among participants aged 45 years or older, we assumed that participants younger than 45 years were cognitively healthy (24), and therefore they were assigned a score of '2.'

Dichotomization lifestyle factors regression analysis

Lifestyle was defined by the following variables: sleep, physical activity, body mass index (weight and height were assessed by a health care worker and were used to calculate BMI (weight (kg) [height (m)]$^{-2}$), smoking status and alcohol consumption. As these behaviors may vary over time, we accounted for this by creating dummy variables that reflected whether someone was engaged in the behavior every measurement round or not. This resulted for smoking status in the following dummy variable: non-smoker in every round versus the rest. For sleeping we took a cut-off point of 7 hours or more sleep duration per 24 hours period. Sufficient physical activity was defined as adherence to the Dutch physical activity guidelines, which recommends 30 minutes of moderate to vigorous physical activity per day on at least 5 days per week (11). BMI was dichotomized as being obese or not (BMI $\geq$ 30.0). We classified someone as an alcohol consumer when the respondent indicated to consume alcohol sometimes or regularly.

C.2 Supplementary figures and tables

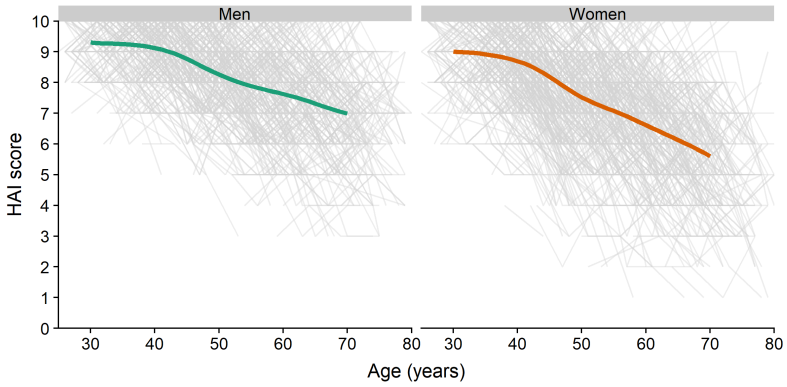

Figure C.1: Development over time of the average HAI scores for men and women

Table C.1: Response rates in the DCS of the included rounds

Period	Invited	Participated	Response	DCS round	This study
1987 – 1991	20154	12404	0.62	1	-
1993 – 1997	7768	6117	0.79	2	T1
1998 – 2002	6581	4918	0.75	3	T2
2003 – 2007	5783	4520	0.78	4	T3
2008 – 2012	5136	4018	0.78	5	T4

Table C.2: Scores at T1 for respondents who had data on all four measurements for the indicator

	Women		Men		Total
	Mean (SD)	Range	Mean (SD)	Range	N
Systolic blood pressure (mm Hg)	119.9 (15.9)	79–200	127.1 (14.6)	93–191	3439
Random glucose ($mmol * L^{-1}$)	5.1 (1.0)	1–17.2	5.4 (1.3)	1–18.3	3167
Creatinine	73.5 (12.6)	37–125	72.8 (12.5)	41–129	3064
FVC (L)	4.0 (0.6)	1.9–7.4	5.4 (1.0)	2.7–27.2	2303
Global cognitive function - T1	0.1 (0.7)	-2.2 – 1.9	0.1 (0.7)	-2.2 – 1.9	403
Global cognitive function - T2	0.0 (0.7)	-2.5 – 1.9	0.0 (0.7)	-2.6 – 2.2	1,390

Note:
Cognition was only measured in a subsample at T1

Table C.3: Scores at T4 for respondents who had data on all four measurements for the indicator

	Women		Men		Total
	Mean (SD)	Range	Mean (SD)	Range	N
Systolic blood pressure (mm Hg)	128.5 (18.0)	84-215	134.8 (17.5)	85-240	3439
Random glucose ($mmol * L^{-1}$)	5.2 (1.4)	2.1-24.2	5.4 (1.4)	2.4-17.2	3167
Creatinine	76.4 (14.4)	35-148	75.8 (15.0)	42-157	3064
FVC (L)	3.6 (0.6)	1.6-6.7	5.0 (0.9)	2.1-8.5	2303
Global cognitive function	-0.04 (0.8)	-4.3	-0.05 (0.7)	-4.2	1390

Note:
Cognition was only measured in a subsample at T1

Table C.4: Scores at T1 on the healthy aging indicators of the imputed sample

	Women		Men		Total
	Mean (SD)	Range	Mean (SD)	Range	N
Systolic blood pressure (mm Hg)	120.7 (16.2)	79-200	127.4 (14.8)	92-191	4461
Random glucose ($mmol * L^{-1}$)	5.2 (1.2)	1-19.6	5.4 (1.3)	1-18.3	4393
Creatinine	73.4 (12.9)	37-146	73.0 (12.6)	41-131	4330
FVC (L)	3.9 (0.6)	1.8-7.4	5.4 (1.0)	2.4-27.2	4002
Global cognitive function- T1	0.1 (0.7)	-2.2 -1.9	0.1 (0.7)	-2.2 - 1.9	403
Global cognitive function - T2	0.0 (0.7)	-2.9 – 1.9	0.0 (0.7)	-2.6 – 2.2	3,403

Note:
Cognition was only measured in a subsample at T1

Table C.5: Scores at T4 on the healthy aging indicators of the imputed sample

	Women		Men		Total
	Mean (SD)	Range	Mean (SD)	Range	N
Systolic blood pressure (mm Hg)	128.4 (18.0)	84-215	134.6 (17.4)	85-240	3903
Random glucose ($mmol * L^{-1}$)	5.2 (1.3)	2.1-24.2	5.4 (1.6)	1.5-18	3993
Creatinine	76.8 (17.3)	35-389	75.9 (16.1)	42-298	3823
FVC (L)	3.6 (0.7)	1.1-6.7	4.9 (0.9)	2.1-8.5	3518
Global cognitive function	-0.02 (0.8)	-2.5-2.7	-0.02 (0.7)	-2.5-2	3115

Table C.6: Score categories at T1 on the healthy aging indicators for complete and imputed sample

	Complete sample		Imputed sample	
	Women, N(%)	Men, N(%)	Women, N(%)	Men, N(%)
Systolic blood pressure (mm Hg)	1,818 (47.2)	1,624 (52.8)	2,342 (52.5)	2,119 (47.5)
0 (hypertension)	11.6	16.9	12.2	16.2
1 (high – normal)	20.2	33.6	21.4	34.9
2 (optimal)	68.2	49.5	66.4	48.9
Random glucose ($mmol * L^{-1}$)	1,656 (52.2)	1,515 (47.8)	2,301 (52.4)	2,092 (47.6)
0 (diabetes)	1.1	1.3	1.4	1.3
1 (pre stage diabetes)	20.6	30.1	20	32.4
2 (no diabetes)	78.3	68.6	78.6	66.3
Creatinine	1,600 (52.2)	1,464 (47.8)	2,314 (53.4)	2,016 (46.6)
0 (unhealthy)	11.8	0.2	11.8	0.3
1 (pre-stage)	45.2	3.4	44.3	3.5
2 (optimal)	43.1	96.5	44	96.2
FVC (L)	1,216 (52.8)	1,085 (47.2)	2,102 (52.6)	1,896 (47.4)
0 (severe airway obstruction)	3.9	3.9	6.2	7
1 (moderate airway obstruction)	14.4	20.6	17	22
2 (good lung function)	81.7	75.5	80.8	71
Global cognitive function	820 (54.9)	674 (45.1)	1,804 (53.0)	1,599 (47.0)
0 (cognitive frail)	1.1	0.6	3.4	3.8
1 (moderate cognition)	5.5	5.3	18.5	20.3
2 (good cognition)	93.4	94.1	78.1	75.9

Table C.7: Score categories at T4 on the healthy aging indicators for complete and imputed sample

	Complete sample		Imputed sample	
	Women, N(%)	Men, N(%)	Women, N(%)	Men, N(%)
Systolic blood pressure (mm Hg)	1,818 (52.8)	1,624 (47.2)	2,342 (52.5)	2,119 (47.5)
0 (hypertension)	37.2	40.9	38	41.3
1 (high – normal)	23.1	30.3	22.9	30.6
2 (optimal)	39.7	28.8	39.1	28.1
Random glucose ($mmol * L^{-1}$)	1,656 (52.2)	1,515 (47.8)	2,301 (52.4)	2,092 (47.6)
0 (diabetes)	6	7.3	6.1	7.4
1 (pre stage diabetes)	18.2	27.2	18.6	28
2 (no diabetes)	75.7	65.5	75.3	64.6
Creatinine	1,600 (52.2)	1,464 (47.8)	2,314 (53.4)	2,016 (46.6)
0 (unhealthy)	18.8	1.6	18.7	2
1 (pre-stage)	43.5	5.9	42.9	5.8
2 (optimal)	37.7	92.5	38.4	92.2
FVC (L)	1,217 (52.8)	1,086 (47.2)	2,102 (52.5)	1,900 (47.5)
0 (severe airway obstruction)	1.2	1.7	1.3	3.1
1 (moderate airway obstruction)	4.4	8	6	8.8
2 (good lung function)	94.5	90.3	92.8	88.2
Global cognitive function	1,712 (52.5)	1,552 (47.5)	1,804 (53.0)	1,599 (47.0)
0 (cognitive frail)	9.9	8.1	9	7.3
1 (moderate cognition)	35	38	32.2	35.7
2 (good cognition)	55.1	54	58.8	57

Table C.8: Respondent characteristics of the total sample at T1

	Women	Men
	n=3255(53%)	n=2858(47%)
Socio-demographic characteristics		
Mean age (SD)	46.2 (10)	46.8 (10)
Age categories, %		
26-35 yr	17.5	15.4
36-45 yr	32.1	31.2
46-55 yr	28.5	31.4
56-65 yr	21.8	22.1
Educational level, %		
Low	63.8	47.5
Medium	22	29.7
High	14.2	22.9
Employed (yes)	44.4	76
Marital status (married)	81.4	82.1
Subjective health		
Excellent / very good	22.9	28.3
Good / moderate	62.9	59.2
Poor	14.2	12.5
Alcohol consumption (>1 per week)	48.9	76.6
Sleep (<7 hours per night)	13.2	19
Smoking status		
Smoker	30.7	32.2
Ex-smoker	32.4	40.5
Never smoker	36.9	27.3
Physical exercise (no)	51.3	52.8
BMI ($kg * m^{-2}$), mean (SD)	25.7 (4.2)	26.1 (3.2)
Healthy Aging index indicators		
Systolic blood pressure, mmHg	122.3 (17.2)	128.7 (15.7)
Random glucose, $mmol * L^{-1}$	5.3 (1.5)	5.5 (1.5)
Creatinine, $mmol * L^{-1}$	72.9 (13.0)	72.6 (12.8)
Lung function ,FVC L	3.9 (0.6)	5.3 (1.0)
Global cognitive function, z-scores*	0.03 (0.7)	0.03 (0.7)
Healthy Aging Index	8.3 (1.5)	8.5 (1.4)

* Scores of T2 are presented due to small sample size at T1

Appendix D

Supplement to Chapter 5

D.1 Supplementary figures

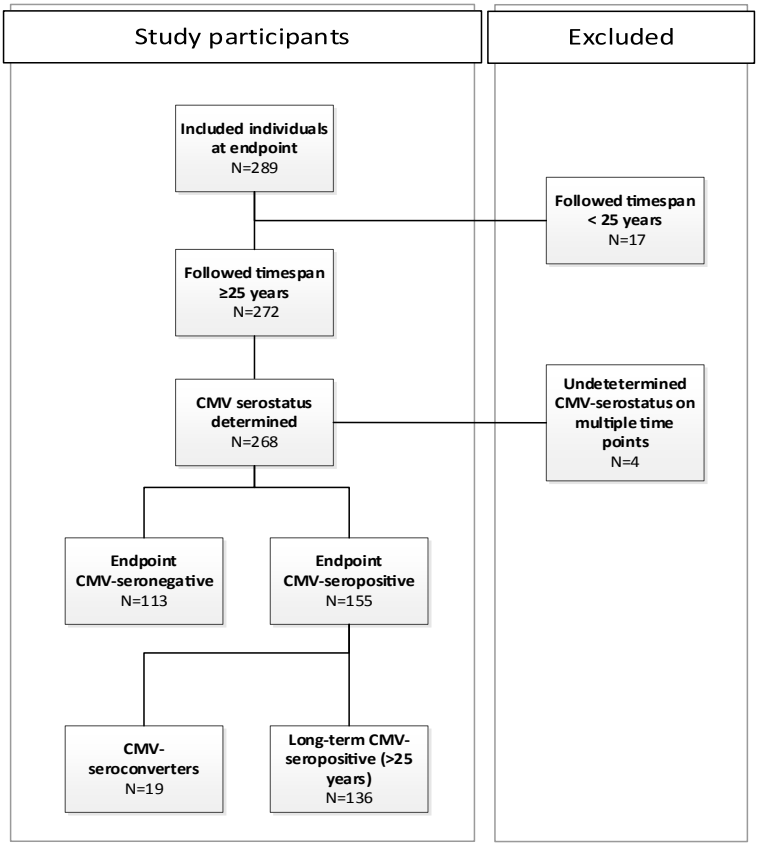

Figure D.1: Flow chart of selection of study subjects.

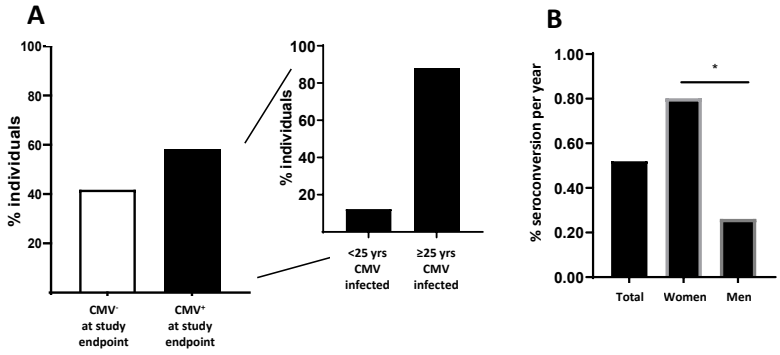

Figure D.2: CMV serostatus at study endpoint and seroconversion rate in the past 27 years. (a) Left panel: CMV serostatus at end point of all individuals. Right panel: duration of CMV infection in CMV+ individuals. (b) Seroconversion rate for total individuals and separated for women and men. Groups difference was investigated with the χ^2 test. Star indicates a P-value < 0.05.

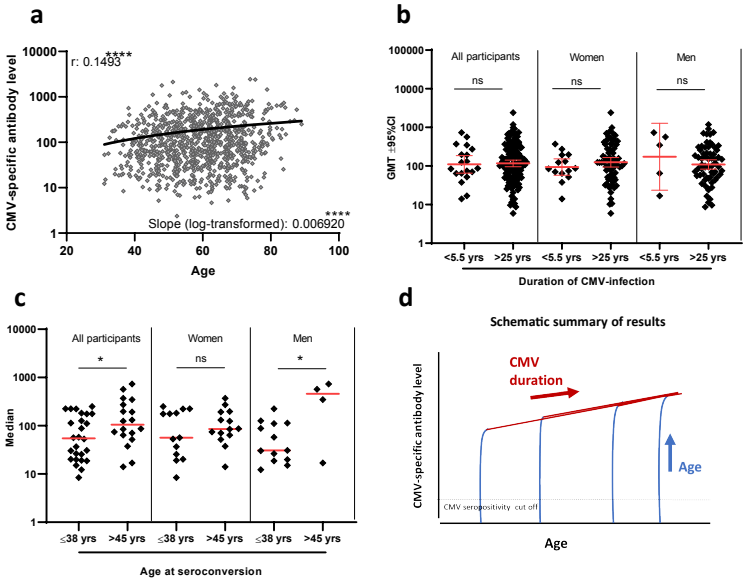

Figure D.3: CMV-specific antibody levels. (a) Results are summarized of the data of CMV-specific antibodies in both long-term CMV$^+$ individuals and CMV seroconverters. (b) Duration of CMV infection: CMV-specific antibody levels of recently seroconverted individuals (max <5.5 year after CMV seroconversion, n = 19) compared with those of long-term CMV+ individuals (n=136, > 25 years CMV$^+$), and similar comparison separately in women and men (middle and right panel, respectively). (c). Age at seroconversion: CMV-specific antibody levels of individuals that seroconverted at younger age ($\leq$ 38yr of age, n = 26) or older age ($\geq$ 45yr of age, n = 18, mean age 58.5$\pm$8.1, shortly after CMV seroconversion (<max 5.5 years)), with similar comparison separately in women and men (middle and right panel, respectively). (d) Schematic summary of results of CMV-specific antibody levels. GMT: geometric mean titer, 95% CI: 95% confidence interval.

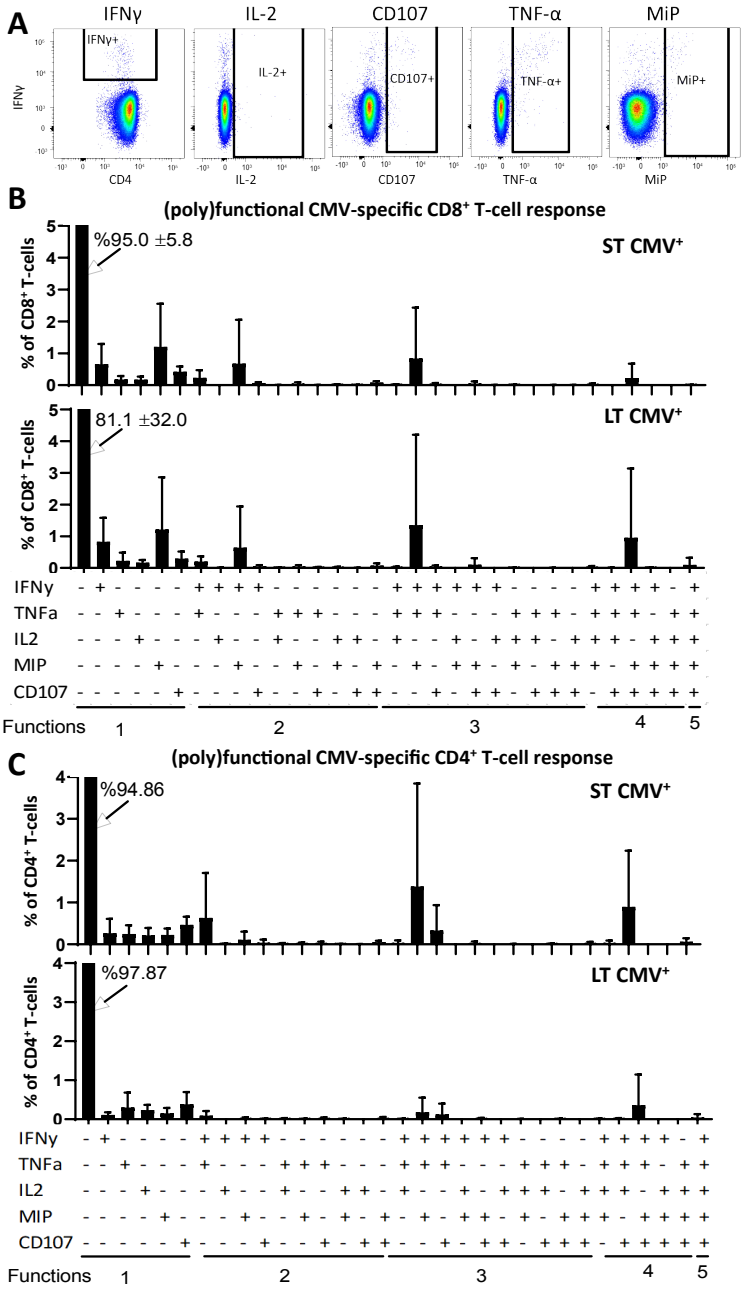

Figure D.4: *(see next page)*

Figure D.4 *(previous page)*: Cytokine profiles after stimulation with CMV-specific peptide pools. (a) Representative flow cytometry plots in the CMV-specific T-cell stimulation assay after stimulation with CMV-specific peptides. (b,c) Percentage of cells that produce IFNγ, TNFα, MIP-1β, CD107, IL-2, or a combination of these cytokines after in vitro stimulation of PBMCs for $CD8^+$ T-cells (b) and (c) $CD4^+$ T-cells.

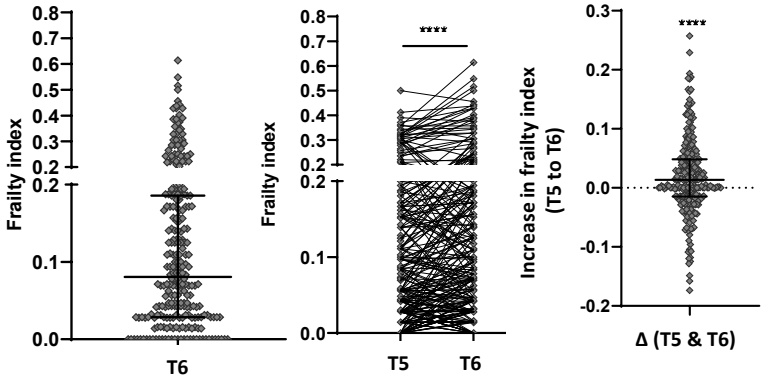

Figure D.5: Frailty index score at time point (T)6 (left panel) and the difference in frailty index score between T5 and T6 (middle and right panel).

Appendix E

Supplement to Chapter 6

E.1 Supplementary figures and tables

Table E.1: Summary characteristics of the cytokines and chemokines in the cohort

Protein	Baseline concentration	Endpoint concentration	AUC
C-reactive protein			
CRP	$1.03(0.93-1.14)*10^6$	$1.22(1.11-1.34)*10^6$	$1.56(1.43-1.70)*10^6$
CC chemokines			
CCL1/I-309	$3.30(3.17-3.43)$	$3.34(3.22-3.46)$	$3.38(3.27-3.50)$
CCL2/MCP-1	$1.03(1.00-1.06)*10^2$	$1.07(1.04-1.10)*10^2$	$1.08(1.06-1.11)*10^2$
CCL5/RANTES	$8.83(8.42-9.26)*10^4$	$7.60(7.27-7.95)*10^4$	$8.00(7.73-8.27)*10^4$
CCL11/Eotaxin	$4.76(4.64-4.89)$	$4.97(4.84-5.11)$	$5.08(4.97-5.19)$
CCL27/C-TACK	$9.34(8.97-9.74)*10^2$	$1.03(0.99-1.08)*10^3$	$1.08(1.05-1.12)*10^3$
CXC chemokines			
CXCL9/MIG	$3.08(2.95-3.23)$	$2.98(2.85-3.11)$	$3.15(3.05-3.26)$
CXCL10/IP-10	$2.37(2.29-2.46)*10^2$	$2.83(2.73-2.93)*10^2$	$3.01(2.92-3.10)*10^2$
CXCL11/I-TAC	$6.07(5.64-6.53)$	$6.19(5.76-6.65)$	$6.98(6.59-7.39)$
Interleukins			
IL-6	$2.43(2.23-2.65)$	$2.31(2.13-2.51)$	$2.61(2.45-2.78)$
IL-10	$0.71(0.66-0.77)$	$0.66(0.62-0.72)$	$0.75(0.71-0.80)$
Soluble receptors			
sCD14	$2.15(2.10-2.21)*10^6$	$2.21(2.15-2.27)*10^6$	$2.27(2.22-2.32)*10^6$
sCD40L	$4.14(3.90-4.40)*10^2$	$3.69(3.48-3.91)*10^2$	$4.05(3.87-4.24)*10^2$
sIL-6R	$2.27(2.22-2.32)*10^4$	$2.35(2.30-2.41)*10^4$	$2.37(2.33-2.42)*10^4$
Other			
P selectin	$7.95(7.43-8.50)*10^4$	$6.80(6.40-7.22)*10^4$	$8.04(7.73-8.36)*10^4$
sGP130	$2.19(2.16-2.22)*10^4$	$2.23(2.20-2.26)*10^4$	$2.25(2.23-2.27)*10^4$
C5a	$4.65(4.38-4.93)*10^3$	$4.90(4.62-5.18)*10^3$	$5.06(4.79-5.34)*10^3$
BDNF	$1.85(1.79-1.91)*10^4$	$1.69(1.63-1.77)*10^4$	$1.75(1.69-1.81)*10^4$

Note:
Concentrations ($pg*mL^{-1}$) and AUC values ($years*pg*mL^{-1}$) are geometric mean values with 95% confidence intervals.

Table E.2: Associations between the inflammatory marker AUC levels and the change in frailty index score between the last two Doetinchem cohort study rounds

Inflammatory marker	n	P value	ρ	FDR	Selected
Analyzed in men					
CRP	52	0.03	0.43	0.47	No
CCL27/C-TACK	52	0.07	0.13	0.63	No
P selectin	52	0.11	-0.22	0.64	No
IL-6	47	0.28	-0.04	1.24	No
CXCL11/I-TAC	52	0.45	-0.07	1.63	No
sCD14	52	0.45	0.10	1.36	No
C5a	52	0.47	0.12	1.22	No
CCL11/Eotaxin	52	0.48	0.15	1.09	No
CXCL9/MIG	52	0.49	0.05	0.98	No
sGP130	47	0.60	-0.13	1.09	No
CXCL10/IP-10	52	0.64	-0.04	1.05	No
IL-10	47	0.70	-0.02	1.05	No
CCL1/I-309	52	0.77	0.10	1.07	No
CCL5/RANTES	52	0.82	0.09	1.06	No
sCD40L	52	0.87	-0.02	1.04	No
sIL-6R	52	0.89	-0.01	1.00	No
BDNF	52	0.95	0.01	1.00	No
CCL2/MCP-1	52	0.99	0.13	0.99	No
Analyzed in women					
sCD40L	58	0.02	-0.25	0.32	No
sCD14	58	0.05	0.31	0.41	No
IL-10	52	0.06	-0.36	0.38	No
IL-6	52	0.06	-0.37	0.29	No
CRP	58	0.09	0.18	0.33	No
P selectin	58	0.18	-0.14	0.54	No
CCL5/RANTES	58	0.18	-0.20	0.47	No
CCL11/Eotaxin	58	0.19	-0.16	0.42	No
BDNF	58	0.27	-0.23	0.54	No
CXCL9/MIG	58	0.33	-0.21	0.59	No
sIL-6R	58	0.36	0.06	0.59	No

CXCL11/I-TAC	58	0.37	-0.14	0.55	No
C5a	58	0.47	0.16	0.66	No
CCL1/I-309	58	0.70	-0.17	0.90	No
CCL2/MCP-1	58	0.79	-0.01	0.95	No
CXCL10/IP-10	58	0.80	0.04	0.90	No
sGP130	53	0.90	0.04	0.95	No
CCL27/C-TACK	58	1.00	0.00	1.00	No

Note:
Permutation version of the Spearman test was used. Analyses are all stratified by initial frailty index score (in tertiles) and by age category (65-70yrs and 70-75yrs). FDR: False Discovery Rate

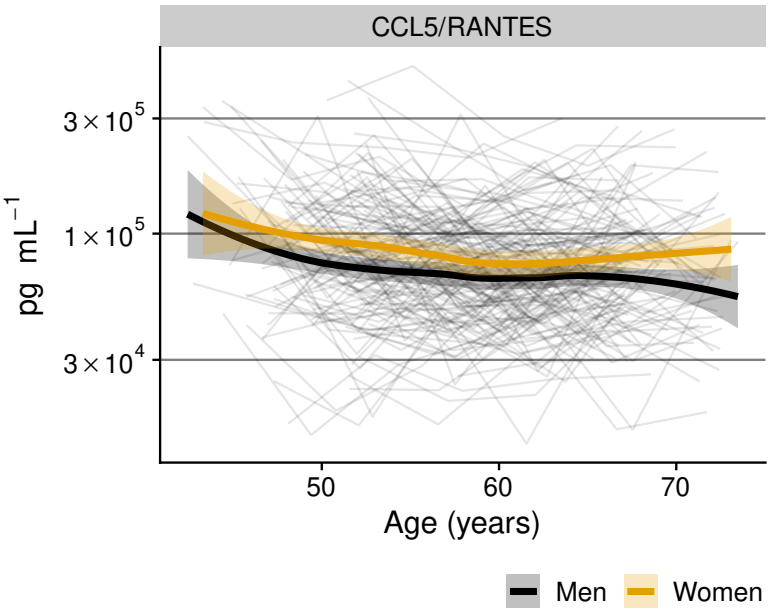

Figure E.1: The concentration of CCL5/RANTES over 20 years in men and women (showing that this is continuously higher in women).

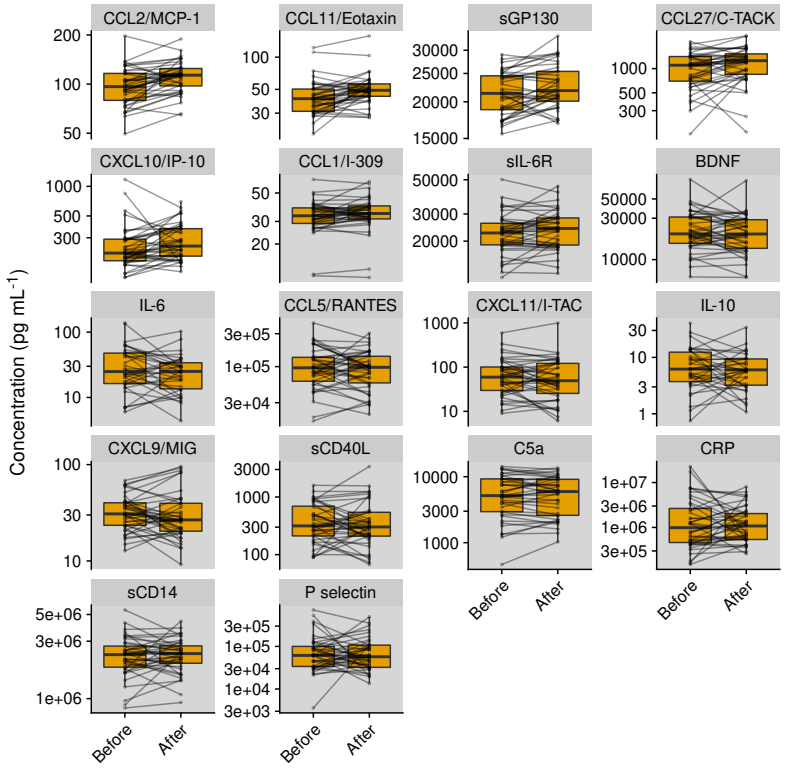

Figure E.2: Menopause is related to changes in inflammatory marker profile. Inflammatory marker concentrations are shown shortly before and shortly after menopause (average difference: 5.3 years) in women of whom data at both timepoints are available (n=40/70). Tiles of biomarkers in which an association was found with menopause are shown in a white background, others in a grey background.

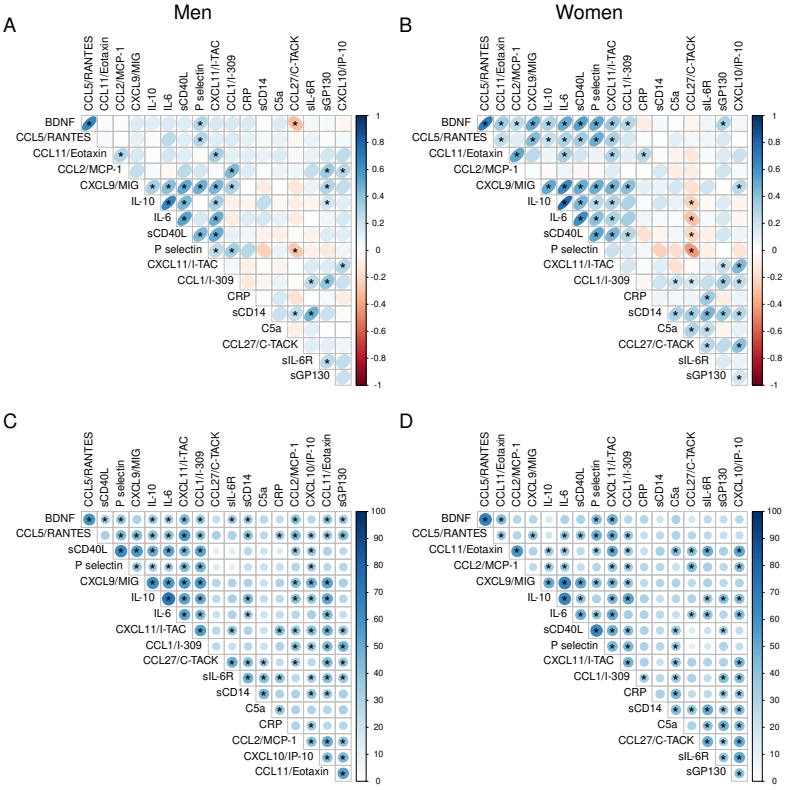

Figure E.3: Relationships between inflammatory markers shown as (A,B) correlation between pairs of inflammatory markers at study endpoint and (C,D) similarity between pairs of inflammatory marker trajectories during about 20years of follow-up. In (A,B) the direction and strength of the association in is visualized with an oval shape and a color gradient. In (C,D) the blue gradient color and the size of the circles shows the percentage of participants of which a pair of biomarkers had the highest increase in concentration at the same moment in 20 years of follow-up. *= an association between two inflammatory markers, with false discovery rate being set at a maximum of 15%. n=71 women, n=73 men.

Appendix F

Supplement to Chapter 7

F.1 Supplementary figures and tables

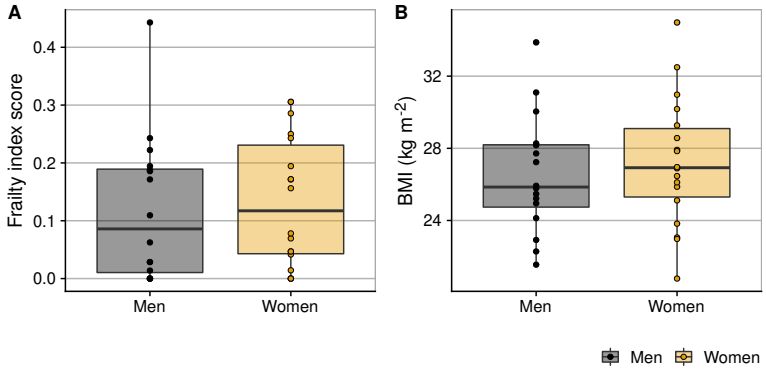

Figure F.1: Frailty index score (A) and BMI values (B) in men and women in the study population (n=16 men, n=18 women).

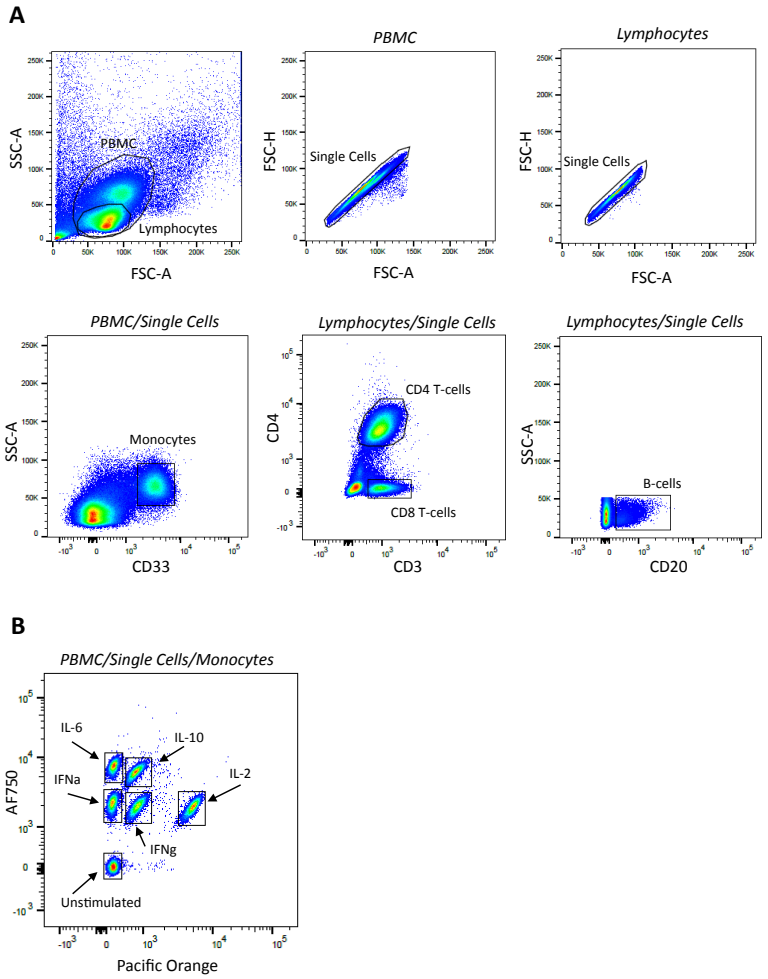

Figure F.2: (A) Phospho-flow cytometry gating strategy, showing gating of leukocytes and lymphocytes, single cells, monocytes, B cells, CD4 T cells and CD8 T cells. (B) Gating strategy to separate different stimulus conditions after barcoding and pooling samples. Gating shown is within monocytes and is representative for the strategy within the other investigated cell subsets.

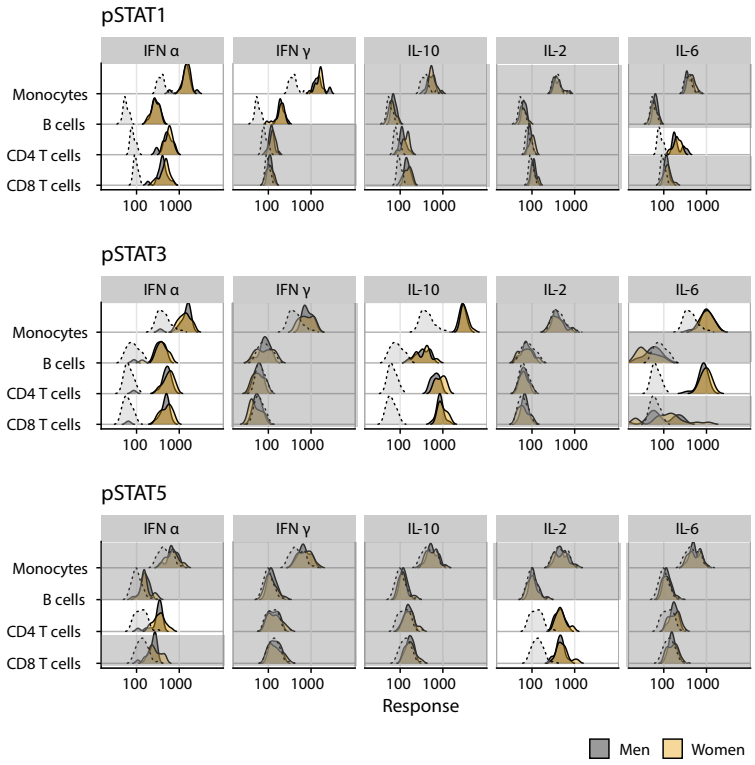

Figure F.3: Phosphorylated STAT (pSTAT1, pSTAT3, pSTAT5) expression at baseline (unstimulated, light-grey densities with dashed lines) and after stimulation in men (dark grey) and women (yellow) with IFNα, IFNγ, IL-10, IL-2 or IL-6, in monocytes, B cells, CD4$^+$ T cells, CD8$^+$ T cells. Selected conditions for further analysis (median fold change>2) are shown with a white background, other plots (non-selected conditions) are presented with a dark grey background.

Table F.1: Selected phosflow conditions with fold change > 2

		Fold change		
Stimulus	pSTAT	Combined (n=32)	Men (n=16)	Women (n=18)
Monocytes				
IFNα	pSTAT1	3.9	4.0	3.9
IFNγ	pSTAT1	3.9	3.9	3.8
IFNα	pSTAT3	3.4	3.4	3.2
IL-10	pSTAT3	7.4	7.0	7.9
IL-6	pSTAT3	2.3	2.2	2.5
B cells				
IFNα	pSTAT1	4.9	4.9	5.2
IFNγ	pSTAT1	3.5	3.3	3.6
IFNα	pSTAT3	4.1	3.9	4.4
IL-10	pSTAT3	3.6	3.7	3.6
CD4$^+$ T cells				
IFNα	pSTAT1	6.8	6.7	6.9
IL-6	pSTAT1	2.5	2.5	2.3
IFNα	pSTAT3	8.1	7.4	9.0
IL-10	pSTAT3	11.9	10.7	13.4
IL-6	pSTAT3	13.9	12.9	14.8
IFNα	pSTAT5	2.5	2.4	2.6
IL-2	pSTAT5	3.8	3.7	4.1
CD8$^+$ T cells				
IFNα	pSTAT1	4.6	4.1	4.9
IFNα	pSTAT3	7.3	6.9	8.3
IL-10	pSTAT3	15.2	12.6	16.0
IL-2	pSTAT5	3.6	3.2	4.1

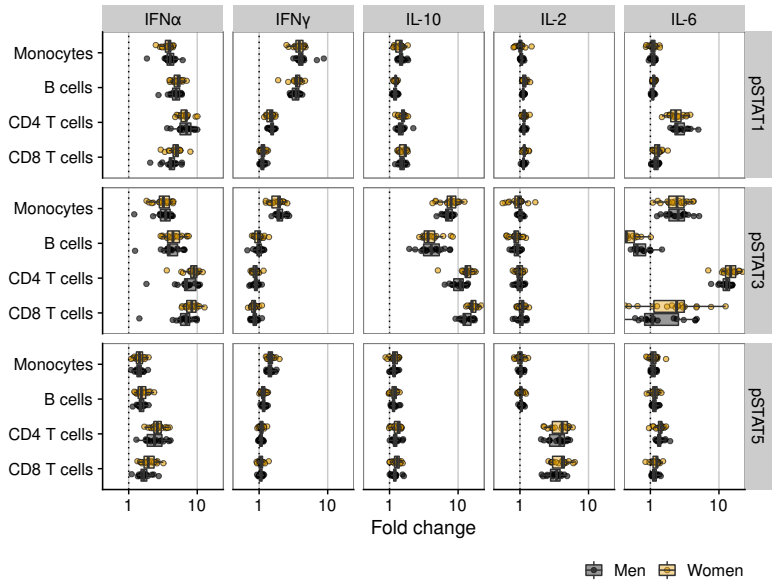

Figure F.4: Fold change (stimulus/baseline) in pSTAT levels (pSTAT1, pSTAT3, pSTAT5) after stimulation with IFNα, IFNγ, IL-10, IL-2 or IL-6, in CD4$^+$ T cells, CD8$^+$ T cells, Monocytes, and B cells in men and women.

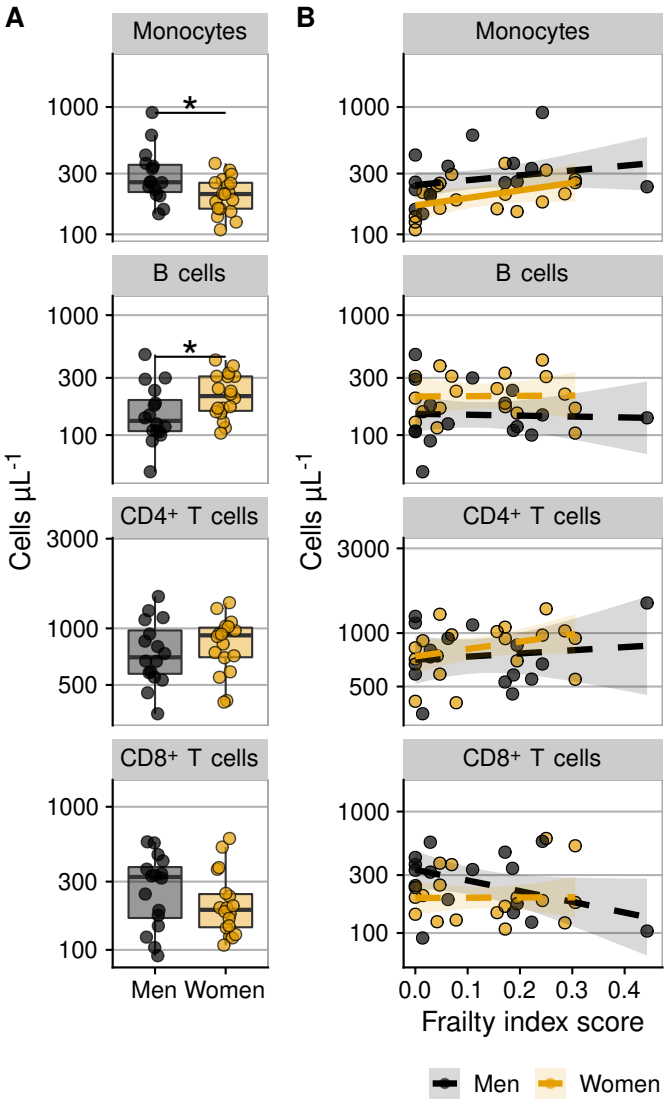

Figure F.5: (A) Association between sex and immune cell numbers. *= association was found with false discovery rate <15%. (B) Association between immune cell numbers and frailty. Continuous trend line means association was found (with false discovery rate <15%), dashed line means that no association was found. Men: n=16, women n=18.

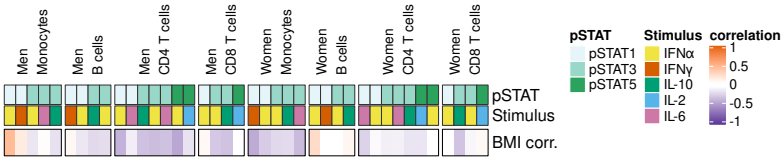

Figure F.6: Heatmap showing the relation between BMI and cellular response to cytokines detected by phosphorylation of STAT1, STAT3, and STAT5 (fold change with baseline levels) in monocytes, B cells, $CD4^+$ T cells and $CD8^+$ T cells of men and women. Every box displays the Spearman's ρ value, based on n=18 women and n=16 men.

Bibliography

Abana, C. O., Pilkinton, M. A., Gaudieri, S., Chopra, A., McDonnell, W. J., Wanjalla, C., Barnett, L., Gangula, R., Hager, C., Jung, D. K., Engelhardt, B. G., Jagasia, M. H., Klenerman, P., Phillips, E. J., Koelle, D. M., Kalams, S. A., & Mallal, S. A. (2017). Cytomegalovirus (CMV) Epitope-Specific CD4(+) T Cells Are Inflated in HIV(+) CMV(+) Subjects. *J Immunol*, *199*(9), 3187–3201. https://doi.org/10.4049/jimmunol.1700851

Abbas, A., Lichtman, AH, & Pillai, S. (2017). Properties and Overview of Immune Responses. In *Cellular and Molecular Immunology* (Ninth, pp. 1–14). Elsevier.

Abnave, P., & Ghigo, E. (2019). Role of the immune system in regeneration and its dynamic interplay with adult stem cells. *Planarian Regeneration*, *87*, 160–168. https://doi.org/10.1016/j.semcdb.2018.04.002

Adler, S. P., & Reddehase, M. J. (2019). Pediatric roots of cytomegalovirus recurrence and memory inflation in the elderly. *Medical Microbiology and Immunology*, *208*(3), 323–328. https://doi.org/10.1007/s00430-019-00609-6

Ahmadi-Abhari, S., Kaptoge, S., Luben, R. N., Wareham, N. J., & Khaw, K.-T. (2014). Longitudinal Association of C-Reactive Protein and Lung Function Over 13 Years: The EPIC-Norfolk Study. *American Journal of Epidemiology*, *179*(1), 48–56. https://doi.org/10.1093/aje/kwt208

Aiello, A. E., Chiu, Y.-L., & Frasca, D. (2017). How does cytomegalovirus factor into diseases of aging and vaccine responses, and by what mechanisms? *GeroScience*, *39*(3), 261–271. https://doi.org/10.1007/s11357-017-9983-9

Aiello, A., Farzaneh, F., Candore, G., Caruso, C., Davinelli, S., Gambino, C. M., Ligotti, M. E., Zareian, N., & Accardi, G. (2019). Immunosenescence and Its Hallmarks: How to Oppose Aging Strategically? A Review of Potential Options for

Therapeutic Intervention. *Frontiers in Immunology*, *10*, 2247. https://doi.org/10.3389/fimmu.2019.02247

Alonso Arias, R., Moro-Garcia, M. A., Echeverria, A., Solano-Jaurrieta, J. J., Suarez-Garcia, F. M., & Lopez-Larrea, C. (2013). Intensity of the humoral response to cytomegalovirus is associated with the phenotypic and functional status of the immune system. *J Virol*, *87*(8), 4486–4495. https://doi.org/10.1128/JVI.02425-12

Aloui, C., Prigent, A., Tariket, S., Sut, C., Fagan, J., Cognasse, F., Chakroun, T., Garraud, O., & Laradi, S. (2016). Levels of human platelet-derived soluble CD40 ligand depend on haplotypes of CD40LG-CD40 - ITGA2. *Scientific Reports*, *6*(1), 24715. https://doi.org/10.1038/srep24715

Altara, R., Gu, Y.-M., Struijker-Boudier, H. A. J., Thijs, L., Staessen, J. A., & Blankesteijn, W. M. (2015). Left Ventricular Dysfunction and CXCR3 Ligands in Hypertension: From Animal Experiments to a Population-Based Pilot Study. *PLOS ONE*, *10*(10), e0141394. https://doi.org/10.1371/journal.pone.0141394

Amanna, I. J., Carlson, N. E., & Slifka, M. K. (2007). Duration of humoral immunity to common viral and vaccine antigens. *N Engl J Med*, *357*(19), 1903–1915. https://doi.org/10.1056/NEJMoa066092

Amici, S. A., Young, N. A., Narvaez-Miranda, J., Jablonski, K. A., Arcos, J., Rosas, L., Papenfuss, T. L., Torrelles, J. B., Jarjour, W. N., & Guerau-de-Arellano, M. (2018). CD38 Is Robustly Induced in Human Macrophages and Monocytes in Inflammatory Conditions. *Frontiers in Immunology*, *9*, 1593. https://doi.org/10.3389/fimmu.2018.01593

Apoil, P. A., Puissant-Lubrano, B., Congy-Jolivet, N., Peres, M., Tkaczuk, J., Roubinet, F., & Blancher, A. (2017). Influence of age, sex and HCMV-serostatus on blood lymphocyte subpopulations in healthy adults. *Cell Immunol*, *314*, 42–53. https://doi.org/10.1016/j.cellimm.2017.02.001

Appay, V., Dunbar, P. R., Callan, M., Klenerman, P., Gillespie, G. M., Papagno, L., Ogg, G. S., King, A., Lechner, F., Spina, C. A., Little, S., Havlir, D. V., Richman, D. D., Gruener, N., Pape, G., Waters, A., Easterbrook, P., Salio, M., Cerundolo, V., … Rowland-Jones, S. L. (2002). Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections. *Nat Med*, *8*(4), 379–385. https://doi.org/10.1038/nm0402-379

Araújo Carvalho, A. C., Tavares Mendes, M. L., Santos, V. S., Tanajura, D. M., Prado Nunes, M. A., & Martins-Filho, P. R. S. (2018). Association between human herpes virus seropositivity and frailty in the elderly: A systematic review and meta-analysis. *Ageing Research Reviews*, *48*, 145–152. https://doi.org/10.1016/j.arr.2018.10.009

Arger, N. K., Ho, M., Woodruff, P. G., & Koth, L. L. (2019). Serum CXCL11 correlates with pulmonary outcomes and disease burden in sarcoidosis. *Respiratory Medicine*, *152*, 89–96. https://doi.org/10.1016/j.rmed.2019.04.005

Aspinall, R., & Lang, P. O. (2018). Interventions to restore appropriate immune function in the elderly. *Immunity & Ageing*, *15*(1), 5. https://doi.org/10.1186/s12979-017-0111-6

Bailey, K. L., Smith, L. M., Heires, A. J., Katafiasz, D. M., Romberger, D. J., & LeVan, T. D. (2019). Aging leads to dysfunctional innate immune responses to TLR2 and TLR4 agonists. *Aging Clinical and Experimental Research*, *31*(9), 1185–1193. https://doi.org/10.1007/s40520-018-1064-0

Baran, P., Hansen, S., Waetzig, G. H., Akbarzadeh, M., Lamertz, L., Huber, H. J., Ahmadian, M. R., Moll, J. M., & Scheller, J. (2018). The balance of interleukin (IL)-6, IL-6·soluble IL-6 receptor (sIL-6R), and IL-6·sIL-6R·sgp130 complexes allows simultaneous classic and trans-signaling. *Journal of Biological Chemistry*, *293*(18), 6762–6775. https://doi.org/10.1074/jbc.RA117.001163

Barnett, K., Mercer, S. W., Norbury, M., Watt, G., Wyke, S., & Guthrie, B. (2012). Epidemiology of multimorbidity and implications for health care, research, and medical education: A cross-sectional study. *The Lancet*, *380*(9836), 37–43. https://doi.org/10.1016/S0140-6736(12)60240-2

Bauman, A., Merom, D., Bull, F. C., Buchner, D. M., & Fiatarone Singh, M. A. (2016). Updating the Evidence for Physical Activity: Summative Reviews of the Epidemiological Evidence, Prevalence, and Interventions to Promote "Active Aging." *The Gerontologist*, *56*(Suppl_2), S268–S280. https://doi.org/10.1093/geront/gnw031

Baylis, Daniel, Bartlett, D. B., Patel, H. P., & Roberts, H. C. (2013). Understanding how we age: Insights into inflammaging. *Longevity & Healthspan*, *2*, 8–8. https://doi.org/10.1186/2046-2395-2-8

Baylis, D., Bartlett, D. B., Syddall, H. E., Ntani, G., Gale, C. R., Cooper, C., Lord, J. M., & Sayer, A. A. (2013). Immune-endocrine biomarkers as predictors of frailty and mortality: A 10-year longitudinal study in community-dwelling older people. *Age (Dordr)*, *35*(3), 963–971. https://doi.org/10.1007/s11357-012-9396-8

Bähler, C., Huber, C. A., Brüngger, B., & Reich, O. (2015). Multimorbidity, health care utilization and costs in an elderly community-dwelling population: A claims data based observational study. *BMC Health Services Research*, *15*(1), 23. https://doi.org/10.1186/s12913-015-0698-2

Beharka, A. A., Meydani, M., Wu, D., Leka, L. S., Meydani, A., & Meydani, S. N. (2001). Interleukin-6 Production Does Not In-

crease With Age. *The Journals of Gerontology: Series A*, *56*(2), B81–B88. https://doi.org/10.1093/gerona/56.2.B81

Benaglia, T., Chauveau, D., Hunter, D. R., & Young, D. (2009). Mixtools : An R Package for Analyzing Finite Mixture Models. *Journal of Statistical Software*, *32*(6). https://doi.org/10.18637/jss.v032.i06

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, *57*(1), 289–300. https://doi.org/10.2307/2346101

Biagi, E., Franceschi, C., Rampelli, S., Severgnini, M., Ostan, R., Turroni, S., Consolandi, C., Quercia, S., Scurti, M., Monti, D., Capri, M., Brigidi, P., & Candela, M. (2016). Gut Microbiota and Extreme Longevity. *Current Biology*, *26*(11), 1480–1485. https://doi.org/10.1016/j.cub.2016.04.016

Bianchi, V. E. (2018). Weight loss is a critical factor to reduce inflammation. *Clinical Nutrition ESPEN*, *28*, 21–35. https://doi.org/10.1016/j.clnesp.2018.08.007

Bowen, M. E., Xuan, L., Lingvay, I., & Halm, E. A. (2015). Random blood glucose: A robust risk factor for type 2 diabetes. *Clin Endocrinol Metab*, *100*(4), 1503–1510. https://doi.org/10.1210/jc.2014-4116

Breiman, L. (2001). Random Forests. *Machine Learning*, *45*(1), 5–32. https://doi.org/10.1023/A:1010933404324

Britanova, O. V., Putintseva, E. V., Shugay, M., Merzlyak, E. M., Turchaninova, M. A., Staroverov, D. B., Bolotin, D. A., Lukyanov, S., Bogdanova, E. A., Mamedov, I. Z., Lebedev, Y. B., & Chudakov, D. M. (2014). Age-Related Decrease in TCR Repertoire Diversity Measured with Deep and Normalized Sequence Profiling. *The Journal of Immunology*, *192*(6), 2689. https://doi.org/10.4049/jimmunol.1302064

Bupp, M. R. G. (2015). Sex, the aging immune system, and chronic disease. *Cellular Immunology*, *294*(2), 102–110. https://doi.org/10.1016/j.cellimm.2015.02.002

Calvani, R., Marini, F., Cesari, M., Buford, T. W., Manini, T. M., Pahor, M., Leeuwenburgh, C., Bernabei, R., Landi, F., & Marzetti, E. (2017). Systemic inflammation, body composition, and physical performance in old community-dwellers. *Journal of Cachexia, Sarcopenia and Muscle*, *8*(1), 69–77. https://doi.org/10.1002/jcsm.12134

Campisi, J., Kapahi, P., Lithgow, G. J., Melov, S., Newman, J. C., & Verdin, E. (2019). From discoveries in ageing research to therapeutics for healthy ageing. *Nature*, *571*(7764), 183–192. https://doi.org/10.1038/s41586-019-1365-2

Cannon, M. J., Hyde, T. B., & Schmid, D. S. (2011). Review of cytomegalovirus shedding in bodily fluids and relevance to congenital cytomegalovirus infection. *Rev Med Virol*, *21*(4), 240–255. https://doi.org/10.1002/rmv.695

Carr, E. J., Dooley, J., Garcia-Perez, J. E., Lagou, V., Lee, J. C., Wouters, C., Meyts, I., Goris, A., Boeckxstaens, G., Linterman, M. A., & Liston, A. (2016). The cellular composition of the human immune system is shaped by age and cohabitation. *Nat Immunol*, *17*(4), 461–468. https://doi.org/10.1038/ni.3371

Ceriello, A., & Colagiuri, S. (2008). International Diabetes Federation guideline for management of postmeal glucose: A review of recommendations. *Diabetic Medicine*, *25*(10), 1151–1156.

Cesari, M., Gambassi, G., van Kan, G. A., & Vellas, B. (2014). The frailty phenotype and the frailty index: Different instruments for different purposes. *Age Ageing*, *43*(1), 10–12. https://doi.org/10.1093/ageing/aft160

Cesari, M., Penninx, B. W. J. H., Pahor, M., Lauretani, F., Corsi, A. M., Williams, G. R., Guralnik, J. M., & Ferrucci, L. (2004). Inflammatory Markers and Physical Performance in Older Persons: The InCHIANTI Study. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *59*(3), M242–M248. https://doi.org/10.1093/gerona/59.3.M242

Chapgier, A., Wynn, R. F., Jouanguy, E., Filipe-Santos, O., Zhang, S., Feinberg, J., Hawkins, K., Casanova, J.-L., & Arkwright, P. D. (2006). Human Complete Stat-1 Deficiency Is Associated with Defective Type I and II IFN Responses In Vitro but Immunity to Some Low Virulence Viruses In Vivo. *The Journal of Immunology*, *176*(8), 5078–5083. https://doi.org/10.4049/jimmunol.176.8.5078

Charlson, M. E., Pompei, P., Ales, K. L., & MacKenzie, C. R. (1987). A new method of classifying prognostic comorbidity in longitudinal studies: Development and validation. *Journal of Chronic Diseases*, *40*(5), 373–383.

Childs, B. G., Gluscevic, M., Baker, D. J., Laberge, R.-M., Marquess, D., Dananberg, J., & van Deursen, J. M. (2017). Senescent cells: An emerging target for diseases of ageing. *Nature Reviews Drug Discovery*, *16*(10), 718–735. https://doi.org/10.1038/nrd.2017.116

Cicin-Sain, L. (2019). Cytomegalovirus memory inflation and immune protection. *Med Microbiol Immunol*, *208*(3-4), 339–347. https://doi.org/10.1007/s00430-019-00607-8

Clegg, A., Young, J., Iliffe, S., Rikkert, M. O., & Rockwood, K. (2013). Frailty in elderly people. *The Lancet*, *381*(9868), 752–762. https://doi.org/10.1016/S0140-6736(12)62167-9

Collerton, J., Martin-Ruiz, C., Davies, K., Hilkens, C. M., Isaacs, J., Kolenda, C., Parker, C., Dunn, M., Catt, M., Jagger, C.,

von Zglinicki, T., & Kirkwood, T. B. (2012). 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*, *133*(6), 456–466. https://doi.org/10.1016/j.mad.2012.05.005

Crow, R. S., Lohman, M. C., Titus, A. J., Cook, S. B., Bruce, M. L., Mackenzie, T. A., Bartels, S. J., & Batsis, J. A. (2019). Association of Obesity and Frailty in Older Adults: NHANES 1999. *The Journal of Nutrition, Health & Aging*, *23*(2), 138–144. https://doi.org/10.1007/s12603-018-1138-x

Damien, P., Cognasse, F., Eyraud, M.-A., Arthaud, C.-A., Pozzetto, B., Garraud, O., & Hamzeh-Cognasse, H. (2015). LPS stimulation of purified human platelets is partly dependent on plasma soluble CD14 to secrete their main secreted product, soluble-CD40-Ligand. *BMC Immunology*, *16*(1). https://doi.org/10.1186/s12865-015-0067-2

Danese, S. (2003). Activated platelets are the source of elevated levels of soluble CD40 ligand in the circulation of inflammatory bowel disease patients. *Gut*, *52*(10), 1435–1441. https://doi.org/10.1136/gut.52.10.1435

Dent, E., Kowal, P., & Hoogendijk, E. O. (2016). Frailty measurement in research and clinical practice: A review. *European Journal of Internal Medicine*, *31*, 3–10.

Dieteren, C. M., Samson, L. D., Schipper, M., van Exel, J., Brouwer, W. B. F., Verschuren, W. M. M., & Picavet, H. S. J. (2020). The Healthy Aging Index analyzed over 15 years in the general population: The Doetinchem Cohort Study. *Preventive Medicine*, 106193. https://doi.org/10.1016/j.ypmed.2020.106193

Dinarello, C. A. (2007). Historical insights into cytokines. *European Journal of Immunology*, *37*(S1), S34–S45. https://doi.org/10.1002/eji.200737772

Dodington, D. W., Desai, H. R., & Woo, M. (2018). JAK/STAT Emerging Players in Metabolism. *Trends in Endocrinology & Metabolism*, *29*(1), 55–65. https://doi.org/10.1016/j.tem.2017.11.001

Duong, L., Radley, H., Lee, B., Dye, D., Pixley, F., Grounds, M., Nelson, D., & Jackaman, C. (2021). Macrophage function in the elderly and impact on injury repair and cancer. *Immunity & Ageing*, *18*(1), 4. https://doi.org/10.1186/s12979-021-00215-2

Dupont, L., & Reeves, M. B. (2016). Cytomegalovirus latency and reactivation: Recent insights into an age old problem. *Rev Med Virol*, *26*(2), 75–89. https://doi.org/10.1002/rmv.1862

Dupuis, S., Jouanguy, E., Al-Hajjar, S., Fieschi, C., Al-Mohsen, I. Z., Al-Jumaah, S., Yang, K., Chapgier, A., Eidenschenk, C., Eid, P.,

Ghonaium, A. A., Tufenkeji, H., Frayha, H., Al-Gazlan, S., Al-Rayes, H., Schreiber, R. D., Gresser, I., & Casanova, J.-L. (2003). Impaired response to interferon-α/β and lethal viral disease in human STAT1 deficiency. *Nature Genetics*, *33*(3), 388–391. https://doi.org/10.1038/ng1097

Ellulu, M. S., Patimah, I., Khaza'ai, H., Rahmat, A., & Abed, Y. (2017). Obesity and inflammation: The linking mechanism and the complications. *Archives of Medical Science*, *4*, 851–863. https://doi.org/10.5114/aoms.2016.58928

Elyahu, Y., Hekselman, I., Eizenberg-Magar, I., Berner, O., Strominger, I., Schiller, M., Mittal, K., Nemirovsky, A., Eremenko, E., Vital, A., Simonovsky, E., Chalifa-Caspi, V., Friedman, N., Yeger-Lotem, E., & Monsonego, A. (2019). Aging promotes reorganization of the CD4 T cell landscape toward extreme regulatory and effector phenotypes. *Science Advances*, *5*(8), eaaw8330. https://doi.org/10.1126/sciadv.aaw8330

Enright, P. L., Johnson, L. R., Connett, J. E., Voelker, H., & Buist, A. S. (1991). Spirometry in the Lung Health Study: 1. Methods and Quality Control. *American Review of Respiratory Disease*, *143*(6), 1215–1223. https://doi.org/10.1164/ajrccm/143.6.1215

Farrell, H. E., Lawler, C., Tan, C. S., MacDonald, K., Bruce, K., Mach, M., Davis-Poynter, N., & Stevenson, P. G. (2016). Murine Cytomegalovirus Exploits Olfaction To Enter New Hosts. *mBio*, *7*(2), e00251–16. https://doi.org/10.1128/mBio.00251-16

Febvre-James, M., Lecureur, V., & Fardel, O. (2020). Potent repression of C-reactive protein (CRP) expression by the JAK1/2 inhibitor ruxolitinib in inflammatory human hepatocytes. *Inflammation Research*, *69*(1), 51–62. https://doi.org/10.1007/s00011-019-01293-1

Ferrucci, L., Corsi, A., Lauretani, F., Bandinelli, S., Bartali, B., Taub, D. D., Guralnik, J. M., & Longo, D. L. (2005). The origins of age-related proinflammatory state. *Blood*, *105*(6), 2294–2299. https://doi.org/10.1182/blood-2004-07-2599

Ferrucci, L., & Fabbri, E. (2018). Inflammageing: Chronic inflammation in ageing, cardiovascular disease, and frailty. *Nature Reviews Cardiology*, *15*(9), 505–522. https://doi.org/10.1038/s41569-018-0064-2

Franceschi, Claudio, Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., & De Benedictis, G. (2006). Inflamm-aging: An Evolutionary Perspective on Immunosenescence. *Annals of the New York Academy of Sciences*, *908*(1), 244–254. https://doi.org/10.1111/j.1749-6632.2000.tb06651.x

Franceschi, C., & Campisi, J. (2014). Chronic inflammation (inflammaging) and its potential contribution to age-associated dis-

eases. *J Gerontol A Biol Sci Med Sci*, *69 Suppl 1*, S4–9. https://doi.org/10.1093/gerona/glu057

Franceschi, Claudio, Garagnani, P., Vitale, G., Capri, M., & Salvioli, S. (2017). Inflammaging and 'Garb-aging.' *Trends in Endocrinology & Metabolism*, *28*(3), 199–212. https://doi.org/10.1016/j.tem.2016.09.005

Fried, L. P., Tangen, C. M., Walston, J., Newman, A. B., Hirsch, C., Gottdiener, J., Seeman, T., Tracy, R., Kop, W. J., Burke, G., McBurnie, M. A., & Cardiovascular Health Study Collaborative Research, G. (2001). Frailty in older adults: Evidence for a phenotype. *J Gerontol A Biol Sci Med Sci*, *56*(3), M146–56. https://doi.org/10.1093/gerona/56.3.M146

Fujimoto, H., Sakata, T., Hamaguchi, Y., Shiga, S., Tohyama, K., Ichiyama, S., Wang, F., & Houwen, B. (2000). Flow cytometric method for enumeration and classification of reactive immature granulocyte populations. *Cytometry*, *42*(6), 371–378. https://doi.org/10.1002/1097-0320(20001215)42:6%3C371::AID-CYTO1004%3E3.0.CO;2-G

Fulop, T., Witkowski, J. M., Olivieri, F., & Larbi, A. (2018). The integration of inflammaging in age-related diseases. *Inflammaging*, *40*, 17–35. https://doi.org/10.1016/j.smim.2018.09.003

Furman, David, Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy, D. W., Fasano, A., Miller, G. W., Miller, A. H., Mantovani, A., Weyand, C. M., Barzilai, N., Goronzy, J. J., Rando, T. A., Effros, R. B., Lucia, A., Kleinstreuer, N., & Slavich, G. M. (2019). Chronic inflammation in the etiology of disease across the life span. *Nature Medicine*, *25*(12), 1822–1832. https://doi.org/10.1038/s41591-019-0675-0

Furman, D., Hejblum, B. P., Simon, N., Jojic, V., Dekker, C. L., Thiebaut, R., Tibshirani, R. J., & Davis, M. M. (2014). Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. *Proceedings of the National Academy of Sciences*, *111*(2), 869–874. https://doi.org/10.1073/pnas.1321060111

Furui, Y., Satake, M., Hoshi, Y., Uchida, S., Suzuki, K., & Tadokoro, K. (2013). Cytomegalovirus (CMV) seroprevalence in Japanese blood donors and high detection frequency of CMV DNA in elderly donors. *Transfusion*, *53*(10), 2190–2197. https://doi.org/10.1111/trf.12390

Gale, C. R., Baylis, D., Cooper, C., & Sayer, A. A. (2013). Inflammatory markers and incident frailty in men and women: The English Longitudinal Study of Ageing. *Age (Dordr)*, *35*(6), 2493–2501. https://doi.org/10.1007/s11357-013-9528-9

Ghigliotti, G., Barisione, C., Garibaldi, S., Fabbi, P., Brunelli, C., Spallarossa, P., Altieri, P., Rosa, G., Spinella, G., Palombo,

D., Arsenescu, R., & Arsenescu, V. (2014). Adipose Tissue Immune Response: Novel Triggers and Consequences for Chronic Inflammatory Conditions. *Inflammation*, *37*(4), 1337–1353. https://doi.org/10.1007/s10753-014-9914-1

Gibson, K. L., Wu, Y.-C., Barnett, Y., Duggan, O., Vaughan, R., Kondeatis, E., Nilsson, B.-O., Wikby, A., Kipling, D., & Dunn-Walters, D. K. (2009). B-cell diversity decreases in old age and is correlated with poor health status. *Aging Cell*, *8*(1), 18–25. https://doi.org/10.1111/j.1474-9726.2008.00443.x

Giuliani, N., Sansoni, P., Girasole, G., Vescovini, R., Passeri, G., Passeri, M., & Pedrazzoni, M. (2001). Serum interleukin-6, soluble interleukin-6 receptor and soluble Gp130 exhibit different patterns of age- and menopause-related changes. *Experimental Gerontology*, *36*(3), 547–557. https://doi.org/10.1016/S0531-5565(00)00220-5

Gkrania-Klotsas, E., Langenberg, C., Sharp, S. J., Luben, R., Khaw, K. T., & Wareham, N. J. (2012). Higher immunoglobulin G antibody levels against cytomegalovirus are associated with incident ischemic heart disease in the population-based EPIC-Norfolk cohort. *J Infect Dis*, *206*(12), 1897–1903. https://doi.org/10.1093/infdis/jis620

Gkrania-Klotsas, E., Langenberg, C., Sharp, S. J., Luben, R., Khaw, K. T., & Wareham, N. J. (2013). Seropositivity and Higher Immunoglobulin G Antibody Levels Against Cytomegalovirus Are Associated With Mortality in the Population-Based European Prospective Investigation of CancerNorfolk Cohort. *Clinical Infectious Diseases*, *56*(10), 1421–1427. https://doi.org/10.1093/cid/cit083

Global Burden of Disease Collaborative Network. (2016). *Global Burden of Disease Study 2016 (GBD 2016) Cause-Specific Mortality 1980-2016. Seattle, United States: Institute for Health Metrics and Evaluation (IHME), 2017.*

Glynn, J. R., & Moss, P. A. H. (2020). Systematic analysis of infectious disease outcomes by age shows lowest severity in school-age children. *Scientific Data*, *7*(1), 329. https://doi.org/10.1038/s41597-020-00668-y

Goldeck, D., Pawelec, G., Norman, K., Steinhagen-Thiessen, E., Oettinger, L., Haehnel, K., & Demuth, I. (2016). No strong correlations between serum cytokine levels, CMV serostatus and hand-grip strength in older subjects in the Berlin BASE-II cohort. *Biogerontology*, *17*(1), 189–198. https://doi.org/10.1007/s10522-015-9577-9

Goldszmid, R. S., & Trinchieri, G. (2012). The price of immunity. *Nature Immunology*, *13*, 932. https://doi.org/10.1038/ni.2422

Gombart, A. F., Pierre, A., & Maggini, S. (2020). A Review of Micronutrients and the Immune SystemWorking in Harmony to Reduce the Risk of Infection. *Nutrients*, *12*(1). https://doi.org/10.3390/nu12010236

Goodwin, K., Viboud, C., & Simonsen, L. (2006). Antibody response to influenza vaccination in the elderly: A quantitative review. *Vaccine*, *24*. https://doi.org/10.1016/j.vaccine.2005.08.105

Gordon, E. H., & Hubbard, R. E. (2019). Do sex differences in chronic disease underpin the sex-frailty paradox? *Mechanisms of Ageing and Development*, *179*, 44–50. https://doi.org/10.1016/j.mad.2019.02.004

Gordon, E. H., Peel, N. M., Samanta, M., Theou, O., Howlett, S. E., & Hubbard, R. E. (2017). Sex differences in frailty: A systematic review and meta-analysis. *Experimental Gerontology*, *89*, 30–40. https://doi.org/10.1016/j.exger.2016.12.021

Gozzetti, A., Capochiani, E., & Bocchia, M. (2020). The Janus kinase 1/2 inhibitor ruxolitinib in COVID-19. *Leukemia*, *34*(10), 2815–2816. https://doi.org/10.1038/s41375-020-01038-8

Greenwell, B. M. (2017). Pdp: An R package for constructing partial dependence plots. *The R Journal*, *9*(1), 421–436.

Greten, F. R., & Grivennikov, S. I. (2019). Inflammation and Cancer: Triggers, Mechanisms, and Consequences. *Immunity*, *51*(1), 27–41. https://doi.org/10.1016/j.immuni.2019.06.025

Gu, Z., Eils, R., & Schlesner, M. (2016). Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics*, *32*(18), 2847–2849. https://doi.org/10.1093/bioinformatics/btw313

Gustafson, C. E., Kim, C., Weyand, C. M., & Goronzy, J. J. (2020). Influence of immune aging on vaccine responses. *Journal of Allergy and Clinical Immunology*, *145*(5), 1309–1321. https://doi.org/10.1016/j.jaci.2020.03.017

Hadley; Averick, J. C., Mara; Bryan. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Hamer Mark, Sabia Severine, Batty G. David, Shipley Martin J., Tabák Adam G., Singh-Manoux Archana, & Kivimaki Mika. (2012). Physical Activity and Inflammatory Markers Over 10 Years. *Circulation*, *126*(8), 928–933. https://doi.org/10.1161/CIRCULATIONAHA.112.103879

Han, H., Ma, Q., Li, C., Liu, R., Zhao, L., Wang, W., Zhang, P., Liu, X., Gao, G., Liu, F., Jiang, Y., Cheng, X., Zhu, C., & Xia, Y. (2020). Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. *Emerging Microbes & Infections*, *9*(1), 1123–1130. https://doi.org/10.1080/22221751.2020.1770129

Hanlon, P., Nicholl, B. I., Jani, B. D., Lee, D., McQueenie, R., & Mair, F. S. (2018). Frailty and pre-frailty in middle-aged and older adults and its association with multimorbidity and mortality: A prospective analysis of 493 737 UK Biobank participants. *The Lancet Public Health*, *3*(7), e323–e332. https://doi.org/10.1016/S2468-2667(18)30091-4

Hearps, A. C., Martin, G. E., Angelovich, T. A., Cheng, W.-J., Maisa, A., Landay, A. L., Jaworowski, A., & Crowe, S. M. (2012). Aging is associated with chronic innate immune activation and dysregulation of monocyte phenotype and function. *Aging Cell*, *11*(5), 867–875. https://doi.org/10.1111/j.1474-9726.2012.00851.x

Hecker, M., Qiu, D., Marquardt, K., Bein, G., & Hackstein, H. (2004). Continuous cytomegalovirus seroconversion in a large group of healthy blood donors. *Vox Sanguinis*, *86*(1), 41–44. https://doi.org/10.1111/j.0042-9007.2004.00388.x

Heijde, D. van der, Strand, V., Tanaka, Y., Keystone, E., Kremer, J., Zerbini, C. A. F., Cardiel, M. H., Cohen, S., Nash, P., Song, Y.-W., Tegzová, D., Gruben, D., Wallenstein, G., Connell, C. A., & Fleischmann, R. (2019). Tofacitinib in Combination With Methotrexate in Patients With Rheumatoid Arthritis: Clinical Efficacy, Radiographic, and Safety Outcomes From a Twenty-FourMonth, Phase III Study. *Arthritis & Rheumatology*, *71*(6), 878–891. https://doi.org/10.1002/art.40803

Herrington, J., Smit, L. S., Schwartz, J., & Carter-Su, C. (2000). The role of STAT proteins in growth hormone signaling. *Oncogene*, *19*(21), 2585–2597. https://doi.org/10.1038/sj.onc.1203526

Hojyo, S., Uchida, M., Tanaka, K., Hasebe, R., Tanaka, Y., Murakami, M., & Hirano, T. (2020). How COVID-19 induces cytokine storm with high mortality. *Inflammation and Regeneration*, *40*(1), 37. https://doi.org/10.1186/s41232-020-00146-3

Homey, B., Alenius, H., Müller, A., Soto, H., Bowman, E. P., Yuan, W., McEvoy, L., Lauerma, A. I., Assmann, T., Bünemann, E., Lehto, M., Wolff, H., Yen, D., Marxhausen, H., To, W., Sedgwick, J., Ruzicka, T., Lehmann, P., & Zlotnik, A. (2002). CCL27CCR10 interactions regulate T cellmediated skin inflammation. *Nature Medicine*, *8*(2), 157–165. https://doi.org/10.1038/nm0202-157

Hosie, L., Pachnio, A., Zuo, J., Pearce, H., Riddell, S., & Moss, P. (2017). Cytomegalovirus-Specific T Cells Restricted by HLA-Cw*0702 Increase Markedly with Age and Dominate the CD8(+) T-Cell Repertoire in Older People. *Front Immunol*, *8*, 1776. https://doi.org/10.3389/fimmu.2017.01776

Hothorn, T., Hornik, K., Wiel, M. A. van de, & Zeileis, A. (2008). Implementing a Class of Permutation Tests: The **Coin** Package. *Journal of Statistical Software*, *28*(8). https://doi.org/10.18637/jss.v028.i08

Hothorn, T., Winell, H., Hornik, K., de Wiel, M. A. van, & Zeileis, A. (2019). *Coin: Conditional inference procedures in a permutation test framework* [Manual].

Hsu, B., Hirani, V., Cumming, R. G., Naganathan, V., Blyth, F. M., Wright, F. C., Waite, L. M., Seibel, M. J., Handelsman, D. J., & Le Couteur, D. G. (2019). Cross-Sectional and Longitudinal Relationships Between Inflammatory Biomarkers and Frailty in Community-dwelling Older Men: The Concord Health and Ageing in Men Project. *The Journals of Gerontology: Series A*, *74*(6), 835–841. https://doi.org/10.1093/gerona/glx142

Huang, J., Jochems, C., Talaie, T., Anderson, A., Jales, A., Tsang, K. Y., Madan, R. A., Gulley, J. L., & Schlom, J. (2012). Elevated serum soluble CD40 ligand in cancer patients may play an immunosuppressive role. *Blood*, *120*(15), 3030–3038. https://doi.org/10.1182/blood-2012-05-427799

Hulsegge, G., Herber-Gast, G. C., Spijkerman, A. M., Susan, H., Picavet, J., van der Schouw, Y. T., Bakker, S. J., Gansevoort, R. T., Dolle, M. E., Smit, H. A., & Monique Verschuren, W. M. (2016). Obesity and Age-Related Changes in Markers of Oxidative Stress and Inflammation Across Four Generations. *Obesity (Silver Spring)*, *24*(6), 1389–1396. https://doi.org/10.1002/oby.21515

Hyde, T. B., Schmid, D. S., & Cannon, M. J. (2010). Cytomegalovirus seroconversion rates and risk factors: Implications for congenital CMV. *Rev Med Virol*, *20*(5), 311–326. https://doi.org/10.1002/rmv.659

Jackson, S. E., Mason, G. M., Okecha, G., Sissons, J. G., & Wills, M. R. (2014). Diverse specificities, phenotypes, and antiviral activities of cytomegalovirus-specific CD8+ T cells. *J Virol*, *88*(18), 10894–10908. https://doi.org/10.1128/JVI.01477-14

Jackson, S. E., Sedikides, G. X., Okecha, G., & Wills, M. R. (2019). Generation, maintenance and tissue distribution of T cell responses to human cytomegalovirus in lytic and latent infection. *Med Microbiol Immunol*. https://doi.org/10.1007/s00430-019-00598-6

Janeway Jr, C. A., Travers, P., Walport, M., & Shlomchik, M. J. (2001). Principles of innate and adaptive immunity. In *Immunobiology: The immune system in health and disease. 5th edition*. Garland Science.

Jenabian, M. A., Patel, M., Kema, I., Vyboh, K., Kanagaratham, C., Radzioch, D., Thébault, P., Lapointe, R., Gilmore, N., Ancuta, P., Tremblay, C., & Routy, J. P. (2014). Soluble CD40-ligand (sCD40L, sCD154) plays an immunosuppressive role via regulatory T cell expansion in HIV infection. *Clinical & Experimental Immunology*, *178*(1), 102–111. https://doi.org/10.1111/cei.12396

Johnstone, J., Parsons, R., Botelho, F., Millar, J., McNeil, S., Fulop, T., McElhaney, J. E., Andrew, M. K., Walter, S. D., Devereaux, P. J., Malek, M., Brinkman, R. R., Bramson, J., & Loeb, M. (2016). T-Cell Phenotypes Predictive of Frailty and Mortality in Elderly Nursing Home Residents. *Journal of the American Geriatrics Society*, n/a–n/a. https://doi.org/10.1111/jgs.14507

Kaczorowski, K. J., Shekhar, K., Nkulikiyimfura, D., Dekker, C. L., Maecker, H., Davis, M. M., Chakraborty, A. K., & Brodin, P. (2017). Continuous immunotypes describe human immune variation and predict diverse responses. *Proceedings of the National Academy of Sciences*, *114*(30), E6097–E6106. https://doi.org/10.1073/pnas.1705065114

Kalil, A. C., Patterson, T. F., Mehta, A. K., Tomashek, K. M., Wolfe, C. R., Ghazaryan, V., Marconi, V. C., Ruiz-Palacios, G. M., Hsieh, L., Kline, S., Tapson, V., Iovine, N. M., Jain, M. K., Sweeney, D. A., Sahly, H. M. E., Branche, A. R., Pineda, J. R., Lye, D. C., Sandkovsky, U., … Beigel, J. H. (2020). Baricitinib plus Remdesivir for Hospitalized Adults with Covid-19. *New England Journal of Medicine*. https://doi.org/10.1056/NEJMoa2031994

Kameda, M., Otsuka, M., Chiba, H., Kuronuma, K., Hasegawa, T., Takahashi, H., & Takahashi, H. (2020). CXCL9, CXCL10, and CXCL11; biomarkers of pulmonary inflammation associated with autoimmunity in patients with collagen vascular diseasesassociated interstitial lung disease and interstitial pneumonia with autoimmune features. *PLOS ONE*, *15*(11), e0241719. https://doi.org/10.1371/journal.pone.0241719

Kemper, H., Ooijendijk, WTM, & Stiggelbout, M. (2000). Consensus over de Nederlandse norm voor gezond bewegen [Consensus on the Dutch standard for healthy exercise]. *Tijdschr Soc Gezondheidsz*, *78*(3), 180–183.

Kennedy, B. K., Berger, S. L., Brunet, A., Campisi, J., Cuervo, A. M., Epel, E. S., Franceschi, C., Lithgow, G. J., Morimoto, R. I., Pessin, J. E., Rando, T. A., Richardson, A., Schadt, E. E., Wyss-Coray, T., & Sierra, F. (2014). Geroscience: Linking Aging to Chronic Disease. *Cell*, *159*(4), 709–713. https://doi.org/10.1016/j.cell.2014.10.039

Khreiss, T., Jozsef, L., Hossain, S., Chan, J. S., Potempa, L. A., & Filep, J. G. (2002). Loss of pentameric symmetry of C-reactive protein is associated with delayed apoptosis of human neutrophils. *J Biol Chem*, *277*(43), 40775–40781. https://doi.org/10.1074/jbc.M205378200

Kim, O., Monsel, A., Bertrand, M., Coriat, P., Cavaillon, J.-M., & Adib-Conquy, M. (2010). Differential down-regulation of HLA-

DR on monocyte subpopulations during systemic inflammation. *Critical Care*, *14*(2), R61. https://doi.org/10.1186/cc8959

Klein, S. L., & Flanagan, K. L. (2016). Sex differences in immune responses. *Nature Reviews Immunology*, *16*(10), 626–638. https://doi.org/10.1038/nri.2016.90

Klenerman, P. (2018). The (gradual) rise of memory inflation. *Immunol Rev*, *283*(1), 99–112. https://doi.org/10.1111/imr.12653

Klenerman, P., & Oxenius, A. (2016). T cell responses to cytomegalovirus. *Nat Rev Immunol*. https://doi.org/10.1038/nri.2016.38

Knights, D., Costello, E. K., & Knight, R. (2011). Supervised classification of human microbiota. *FEMS Microbiology Reviews*, *35*(2), 343–359. https://doi.org/10.1111/j.1574-6976.2010.00251.x

Komsta, L. (2019). *Mblm: Median-based Linear Models*.

Korndewal, M. J., Mollema, L., Tcherniaeva, I., van der Klis, F., Kroes, A. C. M., Oudesluys-Murphy, A. M., Vossen, A. C. T. M., & de Melker, H. E. (2015). Cytomegalovirus infection in the Netherlands: Seroprevalence, risk factors, and implications. *Journal of Clinical Virology*, *63*, 53–58. https://doi.org/10.1016/j.jcv.2014.11.033

Kovtonyuk, L. V., Fritsch, K., Feng, X., Manz, M. G., & Takizawa, H. (2016). Inflamm-Aging of Hematopoiesis, Hematopoietic Stem Cells, and the Bone Marrow Microenvironment. *Front Immunol*, *7*(502), 502. https://doi.org/10.3389/fimmu.2016.00502

Krutzik, P. O., & Nolan, G. P. (2006). Fluorescent cell barcoding in flow cytometry allows high-throughput drug screening and signaling profiling. *Nature Methods*, *3*(5), 361–368. https://doi.org/10.1038/nmeth872

Krzywinski, M., & Altman, N. (2014). Analysis of variance and blocking. *Nature Methods*, *11*(7), 699–700. https://doi.org/10.1038/nmeth.3005

Kulminski, A. M., Ukraintseva, S. V., Kulminskaya, I. V., Arbeev, K. G., Land, K., & Yashin, A. I. (2008). Cumulative deficits better characterize susceptibility to death in elderly people than phenotypic frailty: Lessons from the Cardiovascular Health Study. *J Am Geriatr Soc*, *56*(5), 898–903. https://doi.org/10.1111/j.1532-5415.2008.01656.x

La Rosée, F., Bremer, H. C., Gehrke, I., Kehr, A., Hochhaus, A., Birndt, S., Fellhauer, M., Henkes, M., Kumle, B., Russo, S. G., & La Rosée, P. (2020). The Janus kinase 1/2 inhibitor ruxolitinib in COVID-19 with severe systemic hyperinflammation. *Leukemia*, *34*(7), 1805–1815. https://doi.org/10.1038/s41375-020-0891-0

Lakschevitz, F. S., Hassanpour, S., Rubin, A., Fine, N., Sun, C., & Glogauer, M. (2016). Identification of neutrophil surface marker

changes in health and inflammation using high-throughput screening flow cytometry. *Experimental Cell Research*, *342*(2), 200–209. https://doi.org/10.1016/j.yexcr.2016.03.007

Lebedeva, A. M., Shpektor, A. V., Vasilieva, E. Yu., & Margolis, L. B. (2018). Cytomegalovirus infection in cardiovascular diseases. *Biochemistry (Moscow)*, *83*(12), 1437–1447. https://doi.org/10.1134/S0006297918120027

Lee, J. W., Lee, J. B., Park, M., & Song, S. H. (2005). An extensive comparison of recent classification tools applied to microarray data. *Computational Statistics & Data Analysis*, *48*(4), 869–885. https://doi.org/10.1016/j.csda.2004.03.017

Leins, H., Mulaw, M., Eiwen, K., Sakk, V., Liang, Y., Denkinger, M., Geiger, H., & Schirmbeck, R. (2018). Aged murine hematopoietic stem cells drive aging-associated immune remodeling. *Blood*, *132*(6), 565–576. https://doi.org/10.1182/blood-2018-02-831065

Leng, S. X., Xue, Q. L., Tian, J., Huang, Y., Yeh, S. H., & Fried, L. P. (2009). Associations of neutrophil and monocyte counts with frailty in community-dwelling disabled older women: Results from the Women's Health and Aging Studies I. *Exp Gerontol*, *44*(8), 511–516. https://doi.org/10.1016/j.exger.2009.05.005

Li, M., Yao, D., Zeng, X., Kasakovski, D., Zhang, Y., Chen, S., Zha, X., Li, Y., & Xu, L. (2019). Age related human T cell subset evolution and senescence. *Immunity & Ageing*, *16*(1), 24. https://doi.org/10.1186/s12979-019-0165-8

Liaw, A., & Wiener, M. (2002). Classification and regression by randomForest. *R News*, *2*(3), 18–22.

Libby, P. (2006). Inflammation and cardiovascular disease mechanisms. *The American Journal of Clinical Nutrition*, *83*(2), 456S–460S. https://doi.org/10.1093/ajcn/83.2.456S

Liu, Y. Z., Wang, Y. X., & Jiang, C. L. (2017 received 20). Inflammation: The Common Pathway of Stress-Related Diseases. *Front Hum Neurosci*, *11*, 316. https://doi.org/10.3389/fnhum.2017.00316

Lloberas, J., Tur, J., Vico, T., & Celada, A. (2019). Molecular and Cellular Aspects of Macrophage Aging. In T. Fulop, C. Franceschi, K. Hirokawa, & G. Pawelec (Eds.), *Handbook of Immunosenescence: Basic Understanding and Clinical Implications* (pp. 1631–1663). Springer International Publishing. https://doi.org/10.1007/978-3-319-99375-1_46

Lloyd-Jones, K. L., Kelly, M. M., & Kubes, P. (2008). Varying Importance of Soluble and Membrane CD14 in Endothelial Detection of Lipopolysaccharide. *The Journal of Immunology*, *181*(2), 1446–1453. https://doi.org/10.4049/jimmunol.181.2.1446

Loef, B., van Baarle, D., van der Beek, A. J., van Kerkhof, L. W., van de Langenberg, D., & Proper, K. I. (2016). Klokwerk + study

protocol: An observational study to the effects of nightshift work on body weight and infection susceptibility and the mechanisms underlying these health effects. *BMC Public Health*, *16*(1), 692. https://doi.org/10.1186/s12889-016-3317-1

Lu, L., Zhang, H., Dauphars, D. J., & He, Y.-W. (2020). A Potential Role of Interleukin 10 in COVID-19 Pathogenesis. *Trends in Immunology*. https://doi.org/10.1016/j.it.2020.10.012

Lu, Y., Biancotto, A., Cheung, F., Remmers, E., Shah, N., McCoy, J. P., & Tsang, J. S. (2016). Systematic Analysis of Cell-to-Cell Expression Variation of T Lymphocytes in a Human Cohort Identifies Aging and Genetic Associations. *Immunity*, *45*(5), 1162–1175. https://doi.org/10.1016/j.immuni.2016.10.025

Lubin, J. H., Colt, J. S., Camann, D., Davis, S., Cerhan, J. R., Severson, R. K., Bernstein, L., & Hartge, P. (2004). Epidemiologic Evaluation of Measurement Data in the Presence of Detection Limits. *Environmental Health Perspectives*, *112*(17), 1691–1696. https://doi.org/10.1289/ehp.7199

Luo, W., Li, Y.-X., Jiang, L.-J., Chen, Q., Wang, T., & Ye, D.-W. (2020). Targeting JAK-STAT Signaling to Control Cytokine Release Syndrome in COVID-19. *Trends in Pharmacological Sciences*, *41*(8), 531–543. https://doi.org/10.1016/j.tips.2020.06.007

Lustig, A., Liu, H. B., Metter, E. J., An, Y., Swaby, M. A., Elango, P., Ferrucci, L., Hodes, R. J., & Weng, N. P. (2017). Telomere Shortening, Inflammatory Cytokines, and Anti-Cytomegalovirus Antibody Follow Distinct Age-Associated Trajectories in Humans. *Front Immunol*, *8*, 1027. https://doi.org/10.3389/fimmu.2017.01027

Marcos-Pérez, D., Sánchez-Flores, M., Maseda, A., Lorenzo-López, L., Millán-Calenti, J. C., Gostner, J. M., Fuchs, D., Pásaro, E., Laffon, B., & Valdiglesias, V. (2018). Frailty in Older Adults Is Associated With Plasma Concentrations of Inflammatory Mediators but Not With Lymphocyte Subpopulations. *Frontiers in Immunology*, *9*, 1056. https://doi.org/10.3389/fimmu.2018.01056

Marmot, M. (2005). Social determinants of health inequalities. *The Lancet*, *365*(9464), 1099–1104.

Matheï, C., Vaes, B., Wallemacq, P., & Degryse, J. (2011). Associations Between Cytomegalovirus Infection and Functional Impairment and Frailty in the BELFRAIL Cohort. *Journal of the American Geriatrics Society*, *59*(12), 2201–2208. https://doi.org/10.1111/j.1532-5415.2011.03719.x

Max Roser, E. O.-O., & Ritchie, H. (2013). Life expectancy. *Our World in Data*.

McCabe, E. L., Larson, M. G., Lunetta, K. L., Newman, A. B., Cheng, S., & Murabito, J. M. (2016). Association of an Index of Healthy

Aging With Incident Cardiovascular Disease and Mortality in a Community-Based Sample of Older Adults. *J Gerontol A Biol Sci Med Sci*, *71*(12), 1695–1701. https://doi.org/10.1093/gerona/glw077

McCullagh, P. N., John. (1989). *Generalized Linear Models* (Second Edition). Boca Raton: Champan and Hall/CRC.

Metsios, G. S., Moe, R. H., & Kitas, G. D. (2020). Exercise and inflammation. *Best Practice & Research Clinical Rheumatology*, *34*(2), 101504. https://doi.org/10.1016/j.berh.2020.101504

Michel, J. J., Griffin, P., & Vallejo, A. N. (2016). Functionally Diverse NK-Like T Cells Are Effectors and Predictors of Successful Aging. *Frontiers in Immunology*, *7*. https://doi.org/10.3389/fimmu.2016.00530

Michel, T., Poli, A., Cuapio, A., Briquemont, B., Iserentant, G., Ollert, M., & Zimmer, J. (2016). Human CD56^{bright} NK Cells: An Update. *The Journal of Immunology*, *196*(7), 2923. https://doi.org/10.4049/jimmunol.1502570

Mitchell, W. A., Lang, P. O., & Aspinall, R. (2010). Tracing thymic output in older individuals: Thymic output and age. *Clinical & Experimental Immunology*, *161*(3), 497–503. https://doi.org/10.1111/j.1365-2249.2010.04209.x

Mitnitski, A. B., Mogilner, A. J., & Rockwood, K. (2001). Accumulation of deficits as a proxy measure of aging. *ScientificWorldJournal*, *1*, 323–336. https://doi.org/10.1100/tsw.2001.58

Miyara, M., Yoshioka, Y., Kitoh, A., Shima, T., Wing, K., Niwa, A., Parizot, C., Taflin, C., Heike, T., Valeyre, D., Mathian, A., Nakahata, T., Yamaguchi, T., Nomura, T., Ono, M., Amoura, Z., Gorochov, G., & Sakaguchi, S. (2009). Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. *Immunity*, *30*(6), 899–911. https://doi.org/10.1016/j.immuni.2009.03.019

Morrisette-Thomas, V., Cohen, A. A., Fülöp, T., Riesco, É., Legault, V., Li, Q., Milot, E., Dusseault-Bélanger, F., & Ferrucci, L. (2014). Inflamm-aging does not simply reflect increases in pro-inflammatory markers. *Mechanisms of Ageing and Development*, *139*, 49–57. https://doi.org/10.1016/j.mad.2014.06.005

Mukherjee, R., Kanti Barman, P., Kumar Thatoi, P., Tripathy, R., Kumar Das, B., & Ravindran, B. (2015). Non-Classical monocytes display inflammatory features: Validation in Sepsis and Systemic Lupus Erythematous. *Scientific Reports*, *5*, 13886.

Naccache, P. H., & Lefebvre, J. S. (2014). A straight neutrophil path to healthy aging? *Blood*, *123*(2), 154–156. https://doi.org/10.1182/blood-2013-11-538256

Nagasawa, M., Zhu, Y., Isoda, T., Tomizawa, D., Itoh, S., Kajiwara, M., Morio, T., Nonoyama, S., Shimizu, N., & Mizutani, S. (2005).

Analysis of serum soluble CD40 ligand (sCD40L) in the patients undergoing allogeneic stem cell transplantation: Platelet is a major source of serum sCD40L. *European Journal of Haematology*, *74*(1), 54–60. https://doi.org/10.1111/j.1600-0609.2004.00342.x

Nathanson, C. A. %J. S. S. (1975). Illness and the feminine role: A theoretical review. *Social Science & Medicine*, *9*(2), 57–62.

Newman, A. B., Boudreau, R. M., Naydeck, B. L., Fried, L. F., & Harris, T. B. (2008). A physiologic index of comorbidity: Relationship to mortality and disability. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *63*(6), 603–609.

Ngo, S. T., Steyn, F. J., & McCombe, P. A. (2014). Gender differences in autoimmune disease. *Front Neuroendocrinol*, *35*(3), 347–369. https://doi.org/10.1016/j.yfrne.2014.04.004

Nooyens, A. C., Baan, C. A., Spijkerman, A. M., & Verschuren, W. M. (2010). Type 2 diabetes and cognitive decline in middle-aged men and women: The Doetinchem Cohort Study. *Diabetes Care*, *33*(9), 1964–1969. https://doi.org/10.2337/dc09-2038

Nooyens, A. C. J., Bueno-de-Mesquita, H. B., van Boxtel, M. P. J., van Gelder, B. M., Verhagen, H., & Verschuren, W. M. M. (2011). Fruit and vegetable intake and cognitive decline in middle-aged men and women: The Doetinchem Cohort Study. *British Journal of Nutrition*, *106*(5), 752–761. https://doi.org/10.1017/S0007114511001024

Nooyens, A. C., van Gelder, B. M., & Verschuren, W. M. (2008). Smoking and cognitive decline among middle-aged men and women: The Doetinchem Cohort Study. *Am J Public Health*, *98*(12), 2244–2250. https://doi.org/10.2105/AJPH.2007.130294

Novartis. (2020). Novartis provides update on RUXCOVID study of ruxolitinib for hospitalized patients with COVID-19. In *Novartis*. https://www.novartis.com/news/media-releases/novartis-provides-update-ruxcovid-study-ruxolitinib-hospitalized-patients-covid-19.

O'Connell, M. D., Marron, M. M., Boudreau, R. M., Canney, M., Sanders, J. L., Kenny, R. A., Kritchevsky, S. B., Harris, T. B., & Newman, A. B. (2018). Mortality in Relation to Changes in a Healthy Aging Index: The Health, Aging, and Body Composition Study. *The Journals of Gerontology: Series A*.

O'Shea, J. J., Schwartz, D. M., Villarino, A. V., Gadina, M., McInnes, I. B., & Laurence, A. (2015). The JAK-STAT pathway: Impact on human disease and therapeutic intervention. *Annual Review of Medicine*, *66*, 311–328. https://doi.org/10.1146/annurev-med-051113-024537

Ortona, E., Pierdominici, M., & Rider, V. (2019). Editorial: Sex Hormones and Gender Differences in Immune Responses. *Frontiers*

in Immunology, *10*, 1076. https://doi.org/10.3389/fimmu.2019.01076

Ouchi, N., Parker, J. L., Lugus, J. J., & Walsh, K. (2011). Adipokines in inflammation and metabolic disease. *Nature Reviews Immunology*, *11*(2), 85–97. https://doi.org/10.1038/nri2921

Pachnio, A., Ciaurriz, M., Begum, J., Lal, N., Zuo, J., Beggs, A., & Moss, P. (2016). Cytomegalovirus Infection Leads to Development of High Frequencies of Cytotoxic Virus-Specific CD4+ T Cells Targeted to Vascular Endothelium. *PLOS Pathogens*, *12*(9), e1005832. https://doi.org/10.1371/journal.ppat.1005832

Parry, H. M., Zuo, J., Frumento, G., Mirajkar, N., Inman, C., Edwards, E., Griffiths, M., Pratt, G., & Moss, P. (2016). Cytomegalovirus viral load within blood increases markedly in healthy people over the age of 70 years. *Immun Ageing*, *13*, 1. https://doi.org/10.1186/s12979-015-0056-6

Patel, A. A., Zhang, Y., Fullerton, J. N., Boelen, L., Rongvaux, A., Maini, A. A., Bigley, V., Flavell, R. A., Gilroy, D. W., Asquith, B., Macallan, D., & Yona, S. (2017). The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. *The Journal of Experimental Medicine*, *214*(7), 1913–1923. https://doi.org/10.1084/jem.20170355

Pawelec, G. (2014). Immunosenenescence: Role of cytomegalovirus. *Experimental Gerontology*, *54*, 1–5. https://doi.org/10.1016/j.exger.2013.11.010

Pedersen, T. L. (2019). *Patchwork: The composer of plots* [Manual].

Pennell, L. M., Galligan, C. L., & Fish, E. N. (2012). Sex affects immunity. *Journal of Autoimmunity*, *38*(2-3), J282–J291. https://doi.org/10.1016/j.jaut.2011.11.013

Piber, D., Olmstead, R., Cho, J. H.-J., Witarama, T., Perez, C., Dietz, N., Seeman, T. E., Breen, E. C., Cole, S. W., & Irwin, M. R. (2019). Inflammaging: Age and Systemic, Cellular, and Nuclear Inflammatory Biology in Older Adults. *The Journals of Gerontology: Series A*, *74*(11), 1716–1724. https://doi.org/10.1093/gerona/glz130

Picavet, H. S. J., Blokstra, A., Spijkerman, A. M. W., & Verschuren, W. M. M. (2017). Cohort Profile Update: The Doetinchem Cohort Study 1987-2017: Lifestyle, health and chronic diseases in a life course and ageing perspective. *Int J Epidemiol*, *46*(6), 1751–1751g. https://doi.org/10.1093/ije/dyx103

Pioli, P. D., Casero, D., Montecino-Rodriguez, E., Morrison, S. L., & Dorshkind, K. (2019). Plasma Cells Are Obligate Effectors of Enhanced Myelopoiesis in Aging Bone Marrow. *Immunity*, *51*(2), 351–366.e6. https://doi.org/10.1016/j.immuni.2019.06.006

Pourgheysari, B., Khan, N., Best, D., Bruton, R., Nayak, L., & Moss, P. A. (2007). The cytomegalovirus-specific CD4+ T-cell response

expands with age and markedly alters the CD4+ T-cell repertoire. *J Virol*, *81*(14), 7759–7765. https://doi.org/10.1128/JVI.01262-06

Proust, C., Jacqmin-Gadda, H., Taylor, J. M., Ganiayre, J., & Commenges, D. (2006). A nonlinear model with latent process for cognitive evolution using multivariate longitudinal data. *Biometrics*, *62*(4), 1014–1024.

Proust-Lima, A. L., V; Diakite. (2019). *Lcmm: Extended Mixed Models Using Latent Classes and Latent Processes. R package version: 1.8.1.* https://cran.r-project.org/package=lcmm>.

Proust-Lima, C., Philipps, V., & Liquet, B. (2017). Estimation of extended mixed models using latent classes and latent processes: The R package lcmm. *Journal of Statistical Software*, *78(2)*, 1–56.

Puzianowska-Kuźnicka, M., Owczarz, M., Wieczorowska-Tobis, K., Nadrowski, P., Chudek, J., Slusarczyk, P., Skalska, A., Jonas, M., Franek, E., & Mossakowska, M. (2016). Interleukin-6 and C-reactive protein, successful aging, and mortality: The PolSenior study. *Immunity & Ageing*, *13*(1), 21. https://doi.org/10.1186/s12979-016-0076-x

Quan, H., Li, B., Couris, C. M., Fushimi, K., Graham, P., Hider, P., Januel, J.-M., & Sundararajan, V. (2011). Updating and validating the Charlson comorbidity index and score for risk adjustment in hospital discharge abstracts using data from 6 countries. *American Journal of Epidemiology*, *173*(6), 676–682.

Quanjer, P. H., Stanojevic, S., Cole, T. J., Baur, X., Hall, G. L., Culver, B. H., Enright, P. L., Hankinson, J. L., Ip, M. S. M., Zheng, J., Stocks, J., & the ERS Global Lung Function Initiative. (2012). Multi-ethnic reference values for spirometry for the 3-yr age range: The global lung function 2012 equations. *European Respiratory Journal*, *40*(6), 1324–1343. https://doi.org/10.1183/09031936.00080312

R Core Team. (2019). *R: A language and environment for statistical computing* [Manual]. R Foundation for Statistical Computing.

Rea, I. M., Gibson, D. S., McGilligan, V., McNerlan, S. E., Alexander, H. D., & Ross, O. A. (2018 received 11). Age and Age-Related Diseases: Role of Inflammation Triggers and Cytokines. *Front Immunol*, *9*, 586. https://doi.org/10.3389/fimmu.2018.00586

Reddehase, M. J. (2019). 'Checks and balances' in cytomegalovirus-host cohabitation. *Med Microbiol Immunol*, *208*(3-4), 259–261. https://doi.org/10.1007/s00430-019-00618-5

Redeker, A., Welten, S. P., & Arens, R. (2014). Viral inoculum dose impacts memory T-cell inflation. *Eur J Immunol*, *44*(4), 1046–1057. https://doi.org/10.1002/eji.201343946

Reuser, M., Bonneux, L. G., & Willekens, F. J. (2009). Smoking Kills, Obesity Disables: A Multistate Approach of the US Health and

Retirement Survey. *Obesity*, *17*(4), 783–789. https://doi.org/10.1038/oby.2008.640

Richmond, J. M., Strassner, J. P., Essien, K. I., & Harris, J. E. (2019). T-cell positioning by chemokines in autoimmune skin diseases. *Immunological Reviews*, *289*(1), 186–204. https://doi.org/10.1111/imr.12762

Riera, N., Canalejo, K., Aixalá, M., Rosso, M., Gaddi, E., Bracco, M. M. de E. de, & Galassi, N. (2003). Detection of CD16low neutrophil subpopulations. *Cytometry Part B: Clinical Cytometry*, *51B*(1), 45–46. https://doi.org/10.1002/cyto.b.10004

Rietman, M. L., Hulsegge, G., Nooyens, A. C., Dollé, M. E., Picavet, H. S. J., Bakker, S. J., Gansevoort, R. T., Spijkerman, A. M., & Verschuren, W. M. (2019). Trajectories of (bio) markers during the development of cognitive frailty in the Doetinchem Cohort Study. *Frontiers in Neurology*, *10*.

Ritchie, H., & Roser, M. (2019). Age structure. *Our World in Data*.

Roberts, E. T., Haan, M. N., Dowd, J. B., & Aiello, A. E. (2010). Cytomegalovirus antibody levels, inflammation, and mortality among elderly Latinos over 9 years of follow-up. *Am J Epidemiol*, *172*(4), 363–371. https://doi.org/10.1093/aje/kwq177

Rockwood, K., Andrew, M., & Mitnitski, A. (2007). A comparison of two approaches to measuring frailty in elderly people. *J Gerontol A Biol Sci Med Sci*, *62*(7), 738–743. https://doi.org/10.1093/gerona/62.7.738

Rockwood, Kenneth, & Howlett, S. E. (2019). Age-related deficit accumulation and the diseases of ageing. *Mechanisms of Ageing and Development*, *180*, 107–116. https://doi.org/10.1016/j.mad.2019.04.005

Rockwood, K., & Mitnitski, A. (2007). Frailty in Relation to the Accumulation of Deficits. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *62*(7), 722–727. https://doi.org/10.1093/gerona/62.7.722

Rockwood, Kenneth, Song, X., MacKnight, C., Bergman, H., Hogan, D. B., McDowell, I., & Mitnitski, A. (2005). A global clinical measure of fitness and frailty in elderly people. *Canadian Medical Association Journal*, *173*(5), 489. https://doi.org/10.1503/cmaj.050051

Rockwood, K., Song, X., & Mitnitski, A. (2011). Changes in relative fitness and frailty across the adult lifespan: Evidence from the Canadian National Population Health Survey. *Canadian Medical Association Journal*, *183*(8), E487–E494. https://doi.org/10.1503/cmaj.101271

Rosso, A. L., Sanders, J. L., Arnold, A. M., Boudreau, R. M., Hirsch, C. H., Carlson, M. C., Rosano, C., Kritchevsky, S. B., & Newman,

A. B. (2014). Multisystem physiologic impairments and changes in gait speed of older adults. *Journals of Gerontology Series A: Biomedical Sciences Medical Sciences*, *70*(3), 319–324.

Rowe, J. W., & Kahn, R. L. (1997). Successful aging. *The Gerontologist*, *37*(4), 433–440.

Samson, L. D., Boots, A. M. H., Ferreira, J. A., Picavet, H. S. J., de Rond, L. G. H., de Zeeuw-Brouwer, M., Verschuren, W. M. M., Buisman, A.-M., & Engelfriet, P. (2020). In-depth immune cellular profiling reveals sex-specific associations with frailty. *Immunity & Ageing*, *17*(1), 20. https://doi.org/10.1186/s12979-020-00191-z

Samson, L. D., Boots, A. M. H., Verschuren, W. M. M., Picavet, H. S. J., Engelfriet, P., & Buisman, A.-M. (2019). Frailty is associated with elevated CRP trajectories and higher numbers of neutrophils and monocytes. *Experimental Gerontology*, *125*, 110674. https://doi.org/10.1016/j.exger.2019.110674

Samson, L. D., Buisman, A.-M., Ferreira, J. A., Picavet, H. S. J., Verschuren, W. M. M., Boots, A. M. H., & Engelfriet, P. (2021). Relation of inflammatory marker trajectories with frailty and aging in a 20-year longitudinal study. *bioRxiv*, 2021.02.10.430670. https://doi.org/10.1101/2021.02.10.430670

Sancho-Shimizu, V., Perez de Diego, R., Jouanguy, E., Zhang, S.-Y., & Casanova, J.-L. (2011). Inborn errors of anti-viral interferon immunity in humans. *Current Opinion in Virology*, *1*(6), 487–496. https://doi.org/10.1016/j.coviro.2011.10.016

Sandborn, W. J., Su, C., Sands, B. E., D'Haens, G. R., Vermeire, S., Schreiber, S., Danese, S., Feagan, B. G., Reinisch, W., Niezychowski, W., Friedman, G., Lawendy, N., Yu, D., Woodworth, D., Mukherjee, A., Zhang, H., Healey, P., & Panés, J. (2017). Tofacitinib as Induction and Maintenance Therapy for Ulcerative Colitis. *New England Journal of Medicine*, *376*(18), 1723–1736. https://doi.org/10.1056/NEJMoa1606910

Sanders, J. L., Boudreau, R. M., Penninx, B. W., Simonsick, E. M., Kritchevsky, S. B., Satterfield, S., Harris, T. B., Bauer, D. C., Newman, A. B., & Health, A. B. C. S. (2012). Association of a Modified Physiologic Index with mortality and incident disability: The Health, Aging, and Body Composition study. *J Gerontol A Biol Sci Med Sci*, *67*(12), 1439–1446. https://doi.org/10.1093/gerona/gls123

Sanders, J. L., Minster, R. L., Barmada, M. M., Matteini, A. M., Boudreau, R. M., Christensen, K., Mayeux, R., Borecki, I. B., Zhang, Q., Perls, T., & Newman, A. B. (2014). Heritability of and mortality prediction with a longevity phenotype: The healthy aging index. *J Gerontol A Biol Sci Med Sci*, *69*(4), 479–485. https://doi.org/10.1093/gerona/glt117

Sapey, E., Greenwood, H., Walton, G., Mann, E., Love, A., Aaronson, N., Insall, R. H., Stockley, R. A., & Lord, J. M. (2014). Phosphoinositide 3-kinase inhibition restores neutrophil accuracy in the elderly: Toward targeted treatments for immunosenescence. *Blood*, *123*(2), 239–248. https://doi.org/10.1182/blood-2013-08-519520

Saule, P., Trauet, J., Dutriez, V., Lekeux, V., Dessaint, J.-P., & Labalette, M. (2006). Accumulation of memory T cells from childhood to old age: Central and effector memory cells in CD4+ versus effector memory and terminally differentiated memory cells in CD8+ compartment. *Mechanisms of Ageing and Development*, *127*(3), 274–281. https://doi.org/10.1016/j.mad.2005.11.001

Savva, G. M., Pachnio, A., Kaul, B., Morgan, K., Huppert, F. A., Brayne, C., Moss, P. A. H., & The Medical Research Council Cognitive Function and Ageing Study. (2013). Cytomegalovirus infection is associated with increased mortality in the older population. *Aging Cell*, *12*(3), 381–387. https://doi.org/10.1111/acel.12059

Schmaltz, H. N., Fried, L. P., Xue, Q.-L., Walston, J., Leng, S. X., & Semba, R. D. (2005). Chronic Cytomegalovirus Infection and Inflammation Are Associated with Prevalent Frailty in Community-Dwelling Older Women. *Journal of the American Geriatrics Society*, *53*(5), 747–754. https://doi.org/doi:10.1111/j.1532-5415.2005.53250.x

Schoufour, J. D., Erler, N. S., Jaspers, L., Kiefte-de Jong, J. C., Voortman, T., Ziere, G., Lindemans, J., Klaver, C. C., Tiemeier, H., Stricker, B., Ikram, A. M., Laven, J. S., Brusselle, G. G., Rivadeneira, F., & Franco, O. H. (2017). Design of a frailty index among community living middle-aged and older people: The Rotterdam study. *Maturitas*, *97*, 14–20. https://doi.org/10.1016/j.maturitas.2016.12.002

Schwarz, G. (1978). Estimating the Dimension of a Model. *The Annals of Statistics*, *6*(2). https://doi.org/10.1214/aos/1176344136

Searle, S. D., Mitnitski, A., Gahbauer, E. A., Gill, T. M., & Rockwood, K. (2008). A standard procedure for creating a frailty index. *BMC Geriatr*, *8*, 24. https://doi.org/10.1186/1471-2318-8-24

Sehgal, P. B., Yang, Y.-M., Yuan, H., & Miller, E. J. (2015). STAT5a/b contribute to sex bias in vascular disease: A neuroendocrine perspective. *JAK-STAT*, *4*(3), 1–20. https://doi.org/10.1080/21623996.2015.1090658

Shaw, A. C., Goldstein, D. R., & Montgomery, R. R. (10/25/online 2013). Age-dependent dysregulation of innate immunity. *Nature Reviews Immunology*, *13*, 875. https://doi.org/10.1038/nri3547

Shen-Orr, S. S., Furman, D., Kidd, B. A., Hadad, F., Lovelace, P., Huang, Y. W., Rosenberg-Hasson, Y., Mackey, S., Grisar, F. A., Pickman, Y., Maecker, H. T., Chien, Y. H., Dekker, C. L., Wu, J.

C., Butte, A. J., & Davis, M. M. (2016). Defective Signaling in the JAK-STAT Pathway Tracks with Chronic Inflammation and Cardiovascular Risk in Aging Humans. *Cell Syst*, *3*(4), 374–384 e4. https://doi.org/10.1016/j.cels.2016.09.009

Shive, C. L., Jiang, W., Anthony, D. D., & Lederman, M. M. (2015). Soluble CD14 is a nonspecific marker of monocyte activation. *AIDS (London, England)*, *29*(10), 1263–1265. https://doi.org/10.1097/QAD.0000000000000735

Siegrist, C. A., & Aspinall, R. (2009). B-cell responses to vaccination at the extremes of age. *Nat Rev Immunol*, *9*(3), 185–194. https://doi.org/10.1038/nri2508

Simpson, R. J., Kunz, H., Agha, N., & Graff, R. (2015). Exercise and the regulation of immune functions. *Prog Mol Biol Transl Sci*, *135*. https://doi.org/10.1016/bs.pmbts.2015.08.001

Smith, L., Yang, L., & Hamer, M. (2019). Handgrip strength, inflammatory markers, and mortality. *Scandinavian Journal of Medicine & Science in Sports*, sms.13433. https://doi.org/10.1111/sms.13433

Song, S., Lam, E. W.-F., Tchkonia, T., Kirkland, J. L., & Sun, Y. (2020). Senescent Cells: Emerging Targets for Human Aging and Age-Related Diseases. *Trends in Biochemical Sciences*, *45*(7), 578–592. https://doi.org/10.1016/j.tibs.2020.03.008

Soysal, P., Stubbs, B., Lucato, P., Luchini, C., Solmi, M., Peluso, R., Sergi, G., Isik, A. T., Manzato, E., Maggi, S., Maggio, M., Prina, A. M., Cosco, T. D., Wu, Y. T., & Veronese, N. (2016). Inflammation and frailty in the elderly: A systematic review and meta-analysis. *Ageing Res Rev*, *31*, 1–8. https://doi.org/10.1016/j.arr.2016.08.006

Sproston, N. R., & Ashworth, J. J. (2018 received 13). Role of C-Reactive Protein at Sites of Inflammation and Infection. *Front Immunol*, *9*, 754. https://doi.org/10.3389/fimmu.2018.00754

Steinmann, G. G., Klaus, B., & Muller-Hermelink, H.-K. (1985). The Involution of the Ageing Human Thymic Epithelium is Independent of Puberty.: A Morphometric Study. *Scandinavian Journal of Immunology*, *22*(5), 563–575. https://doi.org/10.1111/j.1365-3083.1985.tb01916.x

Stowe, R. P., Kozlova, E. V., Yetman, D. L., Walling, D. M., Goodwin, J. S., & Glaser, R. (2007). Chronic herpesvirus reactivation occurs in aging. *Exp Gerontol*, *42*(6), 563–570. https://doi.org/10.1016/j.exger.2007.01.005

Stowe, R. P., Peek, M. K., Cutchin, M. P., & Goodwin, J. S. (2012). Reactivation of herpes simplex virus type 1 is associated with cytomegalovirus and age. *J Med Virol*, *84*(11), 1797–1802. https://doi.org/10.1002/jmv.23397

Suttles, J., & Stout, R. D. (2009). Macrophage CD40 signaling: A pivotal regulator of disease protection and pathogenesis. *Seminars in Immunology*, *21*(5), 257–264. https://doi.org/10.1016/j.smim.2009.05.011

Sylwester, A. W., Mitchell, B. L., Edgar, J. B., Taormina, C., Pelte, C., Ruchti, F., Sleath, P. R., Grabstein, K. H., Hosken, N. A., Kern, F., Nelson, J. A., & Picker, L. J. (2005). Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects. *J Exp Med*, *202*(5), 673–685. https://doi.org/10.1084/jem.20050882

Takahashi, T., Ellingson, M. K., Wong, P., Israelow, B., Lucas, C., Klein, J., Silva, J., Mao, T., Oh, J. E., Tokuyama, M., Lu, P., Venkataraman, A., Park, A., Liu, F., Meir, A., Sun, J., Wang, E. Y., Casanovas-Massana, A., Wyllie, A. L., … Iwasaki, A. (2020). Sex differences in immune responses that underlie COVID-19 disease outcomes. *Nature*, *588*(7837), 315–320. https://doi.org/10.1038/s41586-020-2700-3

Tampubolon, G. (2016). Trajectories of the healthy ageing phenotype among middle-aged and older Britons, 2004. *Maturitas*, *88*, 9–15.

Tang, B. M., Shojaei, M., Teoh, S., Meyers, A., Ho, J., Ball, T. B., Keynan, Y., Pisipati, A., Kumar, A., Eisen, D. P., Lai, K., Gillett, M., Santram, R., Geffers, R., Schreiber, J., Mozhui, K., Huang, S., Parnell, G. P., Nalos, M., … Schughart, K. (2019). Neutrophils-related host factors associated with severe disease and fatality in patients with influenza infection. *Nature Communications*, *10*(1), 3422. https://doi.org/10.1038/s41467-019-11249-y

Tang, X., Huang, Y., Lei, J., Luo, H., & Zhu, X. (2019). The single-cell sequencing: New developments and medical applications. *Cell & Bioscience*, *9*(1), 53. https://doi.org/10.1186/s13578-019-0314-y

Tcherniaeva, I., den Hartog, G., Berbers, G., & van der Klis, F. (2018). The development of a bead-based multiplex immunoassay for the detection of IgG antibodies to CMV and EBV. *J Immunol Methods*, *462*, 1–8. https://doi.org/10.1016/j.jim.2018.07.003

Theil, H. (1950). A rank-invariant method of linear and polynomial regression analysis. *Indigationes Mathematicae*, *12*, 85–91.

Thevaranjan, N., Puchta, A., Schulz, C., Naidoo, A., Szamosi, J. C., Verschoor, C. P., Loukov, D., Schenck, L. P., Jury, J., Foley, K. P., Schertzer, J. D., Larché, M. J., Davidson, D. J., Verdú, E. F., Surette, M. G., & Bowdish, D. M. E. (2017). Age-Associated Microbial Dysbiosis Promotes Intestinal Permeability, Systemic Inflammation, and Macrophage Dysfunction. *Cell Host & Microbe*, *21*(4), 455–466.e4. https://doi.org/10.1016/j.chom.2017.03.002

Thiele, J. R., Habersberger, J., Braig, D., Schmidt, Y., Goerendt, K., Maurer, V., Bannasch, H., Scheichl, A., Woollard, K. J., von

Dobschutz, E., Kolodgie, F., Virmani, R., Stark, G. B., Peter, K., & Eisenhardt, S. U. (2014). Dissociation of pentameric to monomeric C-reactive protein localizes and aggravates inflammation: In vivo proof of a powerful proinflammatory mechanism and a new anti-inflammatory strategy. *Circulation*, *130*(1), 35–50. https://doi.org/10.1161/CIRCULATIONAHA.113.007124

Thomasini, R. L., Pereira, D. S., Pereira, F. S. M., Mateo, E. C., Mota, T. N., Guimaraes, G. G., Pereira, L. S. M., Lima, C. X., Teixeira, M. M., & Teixeira, A. L. J. (2017). Aged-associated cytomegalovirus and Epstein-Barr virus reactivation and cytomegalovirus relationship with the frailty syndrome in older women. *PLoS One*, *12*(7), e0180841. https://doi.org/10.1371/journal.pone.0180841

Tsalamandris, S., Antonopoulos, A. S., Oikonomou, E., Papamikroulis, G.-A., Vogiatzi, G., Papaioannou, S., Deftereos, S., & Tousoulis, D. (2019). The Role of Inflammation in Diabetes: Current Concepts and Future Perspectives. *European Cardiology Review*, *14*(1), 50–59. https://doi.org/10.15420/ecr.2018.33.1

Turner, J. E., Campbell, J. P., Edwards, K. M., Howarth, L. J., Pawelec, G., Aldred, S., Moss, P., Drayson, M. T., Burns, V. E., & Bosch, J. A. (2014). Rudimentary signs of immunosenescence in Cytomegalovirus-seropositive healthy young adults. *Age (Dordr)*, *36*(1), 287–297. https://doi.org/10.1007/s11357-013-9557-4

Tuttle, C. S. L., Thang, L. A. N., & Maier, A. B. (2020). Markers of inflammation and their association with muscle strength and mass: A systematic review and meta-analysis. *Ageing Research Reviews*, *64*, 101185. https://doi.org/10.1016/j.arr.2020.101185

United Nations. (2017). *World Population Ageing 2015*. UN. https://doi.org/10.18356/88fa44e7-en

United Nations, Department of Economic and Social Affairs, & Population Division. (2019). *World population prospects Highlights, 2019 revision Highlights, 2019 revision*.

Vairo, D., Tassone, L., Tabellini, G., Tamassia, N., Gasperini, S., Bazzoni, F., Plebani, A., Porta, F., Notarangelo, L. D., Parolini, S., Giliani, S., & Badolato, R. (2011). Severe impairment of IFN-γ and IFN-α responses in cells of a patient with a novel STAT1 splicing mutation. *Blood*, *118*(7), 1806–1817. https://doi.org/10.1182/blood-2011-01-330571

Van Acker, H. H., Capsomidis, A., Smits, E. L., & Van Tendeloo, V. F. (2017). CD56 in the Immune System: More Than a Marker for Cytotoxicity? *Frontiers in Immunology*, *8*, 892. https://doi.org/10.3389/fimmu.2017.00892

van Beek, J., Veenhoven, R. H., Bruin, J. P., van Boxtel, R. A. J., de Lange, M. M. A., Meijer, A., Sanders, E. A. M., Rots, N. Y.,

& Luytjes, W. (2017). Influenza-like Illness Incidence Is Not Reduced by Influenza Vaccination in a Cohort of Older Adults, Despite Effectively Reducing Laboratory-Confirmed Influenza Virus Infections. *The Journal of Infectious Diseases*, *216*(4), 415–424. https://doi.org/10.1093/infdis/jix268

van Boven, M., van de Kassteele, J., Korndewal, M. J., van Dorp, C. H., Kretzschmar, M., van der Klis, F., de Melker, H. E., Vossen, A. C., & van Baarle, D. (2017). Infectious reactivation of cytomegalovirus explaining age- and sex-specific patterns of seroprevalence. *PLoS Comput Biol*, *13*(9), e1005719. https://doi.org/10.1371/journal.pcbi.1005719

van de Berg, P. J., van Stijn, A., Ten Berge, I. J., & van Lier, R. A. (2008). A fingerprint left by cytomegalovirus infection in the human T cell compartment. *J Clin Virol*, *41*(3), 213–217. https://doi.org/10.1016/j.jcv.2007.10.016

van de Berg, P. J., Yong, S. L., Remmerswaal, E. B., van Lier, R. A., & ten Berge, I. J. (2012). Cytomegalovirus-induced effector T cells cause endothelial cell damage. *Clin Vaccine Immunol*, *19*(5), 772–779. https://doi.org/10.1128/CVI.00011-12

van den Biggelaar, A. H. J., Huizinga, T. W. J., de Craen, A. J. M., Gussekloo, J., Heijmans, B. T., Frölich, M., & Westendorp, R. G. J. (2004). Impaired innate immunity predicts frailty in old age. The Leiden 85-plus study. *Experimental Gerontology*, *39*(9), 1407–1414. https://doi.org/10.1016/j.exger.2004.06.009

van den Heuvel, D., Jansen, M. A. E., Nasserinejad, K., Dik, W. A., van Lochem, E. G., Bakker-Jonges, L. E., Bouallouch-Charif, H., Jaddoe, V. W. V., Hooijkaas, H., van Dongen, J. J. M., Moll, H. A., & van Zelm, M. C. (2017). Effects of nongenetic factors on immune cell dynamics in early childhood: The Generation R Study. *Journal of Allergy and Clinical Immunology*, *139*(6), 1923–1934.e17. https://doi.org/10.1016/j.jaci.2016.10.023

van der Heiden, M., van Zelm, M. C., Bartol, S. J., de Rond, L. G., Berbers, G. A., Boots, A. M., & Buisman, A. M. (2016). Differential effects of Cytomegalovirus carriage on the immune phenotype of middle-aged males and females. *Sci Rep*, *6*, 26892. https://doi.org/10.1038/srep26892

van Deursen, J. M. (2014). The role of senescent cells in ageing. *Nature*, *509*(7501), 439–446. https://doi.org/10.1038/nature13193

Van Epps, P., Oswald, D., Higgins, P. A., Hornick, T. R., Aung, H., Banks, R. E., Wilson, B. M., Burant, C., Gravenstein, S., & Canaday, D. H. (2016). Frailty has a stronger association with inflammation than age in older veterans. *Immunity & Ageing*, *13*(1), 27. https://doi.org/10.1186/s12979-016-0082-z

van Lier, A., Smits, G., Mollema, L., Waaijenborg, S., Berbers, G., van der Klis, F., Boot, H., Wallinga, J., & de Melker, H. (2013).

Varicella zoster virus infection occurs at a relatively young age in the Netherlands. *Vaccine*, *31*(44), 5127–5133. https://doi.org/10.1016/j.vaccine.2013.08.029

van Lier, R. A. W., & Kuijpers, T. W. (2017). The cellular immune system comes of age. *Journal of Allergy and Clinical Immunology*, *139*(6), 1793–1794. https://doi.org/10.1016/j.jaci.2017.02.023

van Oostrom, Sandra H., Engelfriet, P. M., Verschuren, W. M. M., Schipper, M., Wouters, I. M., Boezen, M., Smit, H. A., Kerstjens, H. A. M., & Picavet, H. S. J. (2018). Aging-related trajectories of lung function in the general populationThe Doetinchem Cohort Study. *PLOS ONE*, *13*(5), e0197250. https://doi.org/10.1371/journal.pone.0197250

van Oostrom, Sandra H., Picavet, H. S. J., de Bruin, S. R., Stirbu, I., Korevaar, J. C., Schellevis, F. G., & Baan, C. A. (2014). Multimorbidity of chronic diseases and health care utilization in general practice. *BMC Family Practice*, *15*(1), 61. https://doi.org/10.1186/1471-2296-15-61

Verberk, J. D. M., Vos, R. A., Mollema, L., van Vliet, J., van Weert, J. W. M., de Melker, H. E., & van der Klis, F. R. M. (2019). Third national biobank for population-based seroprevalence studies in the Netherlands, including the Caribbean Netherlands. *BMC Infectious Diseases*, *19*(1), 470. https://doi.org/10.1186/s12879-019-4019-y

Verschuren, W. M., Blokstra, A., Picavet, H. S., & Smit, H. A. (2008). Cohort profile: The Doetinchem Cohort Study. *Int J Epidemiol*, *37*(6), 1236–1241. https://doi.org/10.1093/ije/dym292

Vescovini, R., Biasini, C., Telera, A. R., Basaglia, M., Stella, A., Magalini, F., Bucci, L., Monti, D., Lazzarotto, T., Dal Monte, P., Pedrazzoni, M., Medici, M. C., Chezzi, C., Franceschi, C., Fagnoni, F. F., & Sansoni, P. (2010). Intense antiextracellular adaptive immune response to human cytomegalovirus in very old subjects with impaired health and cognitive and functional status. *J Immunol*, *184*(6), 3242–3249. https://doi.org/10.4049/jimmunol.0902890

Vescovini, R., Telera, A. R., Pedrazzoni, M., Abbate, B., Rossetti, P., Verzicco, I., Arcangeletti, M. C., Medici, M. C., Calderaro, A., Volpi, R., Sansoni, P., & Fagnoni, F. F. (2016). Impact of Persistent Cytomegalovirus Infection on Dynamic Changes in Human Immune System Profile. *PLoS One*, *11*(3), e0151965. https://doi.org/10.1371/journal.pone.0151965

Villarino, A. V., Kanno, Y., & O'Shea, J. J. (2017). Mechanisms and consequences of JakSTAT signaling in the immune system. *Nature Immunology*, *18*(4), 374–384. https://doi.org/10.1038/ni.3691

Vukmanovic-Stejic, M., Chambers, E. S., Suarez-Farinas, M., Sandhu, D., Fuentes-Duculan, J., Patel, N., Agius, E., Lacy, K. E., Turner,

C. T., Larbi, A., Birault, V., Noursadeghi, M., Mabbott, N. A., Rustin, M. H. A., Krueger, J. G., & Akbar, A. N. (2018). Enhancement of cutaneous immunity during aging by blocking P38 mitogen-activated protein (MAP) kinase-induced inflammation. *J Allergy Clin Immunol*, *142*(3), 844–856. https://doi.org/10.1016/j.jaci.2017.10.032

Walker, K. A., Walston, J., Gottesman, R. F., Kucharska-Newton, A., Palta, P., & Windham, B. G. (2018). Midlife Systemic Inflammation is Associated with Frailty in Later Life: The ARIC Study. *J Gerontol A Biol Sci Med Sci*, gly045–gly045. https://doi.org/10.1093/gerona/gly045

Walker, K. A., Windham, B. G., Power, M. C., Hoogeveen, R. C., Folsom, A. R., Ballantyne, C. M., Knopman, D. S., Selvin, E., Jack, C. R., & Gottesman, R. F. (2018). The association of mid-to late-life systemic inflammation with white matter structure in older adults: The Atherosclerosis Risk in Communities Study. *Neurobiol Aging*, *68*, 26–33. https://doi.org/10.1016/j.neurobiolaging.2018.03.031

Walston, J., Hadley, E. C., Ferrucci, L., Guralnik, J. M., Newman, A. B., Studenski, S. A., Ershler, W. B., Harris, T., & Fried, L. P. (2006). Research agenda for frailty in older adults: Toward a better understanding of physiology and etiology: Summary from the American Geriatrics Society/National Institute on Aging Research Conference on Frailty in Older Adults. *Journal of the American Geriatrics Society*, *54*(6), 991–1001. https://doi.org/10.1111/j.1532-5415.2006.00745.x

Wang, G. C., Kao, W. H., Murakami, P., Xue, Q. L., Chiou, R. B., Detrick, B., McDyer, J. F., Semba, R. D., Casolaro, V., Walston, J. D., & Fried, L. P. (2010). Cytomegalovirus infection and the risk of mortality and frailty in older women: A prospective observational cohort study. *Am J Epidemiol*, *171*(10), 1144–1152. https://doi.org/10.1093/aje/kwq062

Wang, H., Peng, G., Bai, J., He, B., Huang, K., Hu, X., & Liu, D. (2017). Cytomegalovirus Infection and Relative Risk of Cardiovascular Disease (Ischemic Heart Disease, Stroke, and Cardiovascular Death): A Meta-Analysis of Prospective Studies Up to 2016. *Journal of the American Heart Association*, *6*(7). https://doi.org/10.1161/JAHA.116.005025

Wang, Q., Westra, J., van der Geest, K. S. M., Moser, J., Bijzet, J., Kuiper, T., Lorencetti, P. G., Joosten, L. A. B., Netea, M. G., Heeringa, P., Brouwer, E., & Boots, A. M. H. (2016). Reduced levels of cytosolic DNA sensor AIM2 are associated with impaired cytokine responses in healthy elderly. *Experimental Gerontology*, *78*, 39–46. https://doi.org/10.1016/j.exger.2016.02.016

Wei, T., & Simko, V. (2017). *R package "corrplot": Visualization of a correlation matrix* [Manual].

Weltevrede, M., Eilers, R., de Melker, H. E., & van Baarle, D. (2016). Cytomegalovirus persistence and T-cell immunosenescence in people aged fifty and older: A systematic review. *Exp Gerontol*. https://doi.org/10.1016/j.exger.2016.02.005

Wen, L., & Tang, F. (2018). Boosting the power of single-cell analysis. *Nature Biotechnology*, *36*(5), 408–409. https://doi.org/10.1038/nbt.4131

Wertheimer, A. M., Bennett, M. S., Park, B., Uhrlaub, J. L., Martinez, C., Pulko, V., Currier, N. L., Nikolich-Zugich, D., Kaye, J., & Nikolich-Zugich, J. (2014). Aging and cytomegalovirus infection differentially and jointly affect distinct circulating T cell subsets in humans. *J Immunol*, *192*(5), 2143–2155. https://doi.org/10.4049/jimmunol.1301721

Weyand, C. M., & Goronzy, J. J. (2016). Aging of the Immune System. Mechanisms and Therapeutic Targets. *Annals of the American Thoracic Society*, *13 Suppl 5*(Suppl 5), S422–S428. https://doi.org/10.1513/AnnalsATS.201602-095AW

Whitson, H. E., Cohen, H. J., Schmader, K. E., Morey, M. C., Kuchel, G., & Colon-Emeric, C. S. (2018). Physical Resilience: Not Simply the Opposite of Frailty. *Journal of the American Geriatrics Society*, *66*(8), 1459–1461. https://doi.org/10.1111/jgs.15233

WHO. (2020a). *Ageing: Healthy ageing and functional ability*. https://www.who.int/westernpacific/news/q-a-detail/ageing-healthy-ageing-and-functional-ability.

WHO. (2020b). *Decade of Healthy Ageing (2020-2030)*. https://www.who.int/initiatives/decade-of-healthy-ageing.

Wickham, H. (2016). *Ggplot2: Elegant graphics for data analysis*. Springer-Verlag New York.

Wickham, H., Chang, W., Henry, L., Pedersen, T. L., Takahashi, K., Wilke, C., Woo, K., & Yutani, H. (2019). *Ggplot2: Create elegant data visualisations using the grammar of graphics* [Manual].

Wickham, H., François, R., Henry, L., & Müller, K. (2020). *Dplyr: A grammar of data manipulation* [Manual].

Wickham, H., & Henry, L. (2020). *Tidyr: Tidy messy data* [Manual].

Wilcox, R. (1998). A Note on the Theil-Sen Regression Estimator When the Regressor Is Random and the Error Term Is Heteroscedastic. *Biometrical Journal*, *40*(3), 261–268. https://doi.org/10.1002/(sici)1521-4036(199807)40:3%3C261::Aid-bimj261%3E3.0.Co;2-v

Wilke, C. O. (2019). *Cowplot: Streamlined plot theme and plot annotations for 'Ggplot2'* [Manual].

Wilkie, R., Tajar, A., & McBeth, J. (2013). The onset of widespread musculoskeletal pain is associated with a decrease in healthy age-

ing in older people: A population-based prospective study. *PLoS One*, *8*(3), e59858. https://doi.org/10.1371/journal.pone.0059858

Wilms, E., Troost, F. J., Elizalde, M., Winkens, B., de Vos, P., Mujagic, Z., Jonkers, D. M. A. E., & Masclee, A. A. M. (2020). Intestinal barrier function is maintained with aging a comprehensive study in healthy subjects and irritable bowel syndrome patients. *Scientific Reports*, *10*(1), 475. https://doi.org/10.1038/s41598-019-57106-2

Wilson, D., Drew, W., Jasper, A., Crisford, H., Nightingale, P., Newby, P., Jackson, T., Lord, J. M., & Sapey, E. (2020). Frailty Is Associated With Neutrophil Dysfunction Which Is Correctable With Phosphoinositol-3-Kinase Inhibitors. *The Journals of Gerontology: Series A*, *75*(12), 2320–2325. https://doi.org/10.1093/gerona/glaa216

Wrammert, J., & Ahmed, R. (2008). Maintenance of serological memory. *Biol Chem*, *389*(5), 537–539. https://doi.org/10.1515/bc.2008.066

Wu, C., Smit, E., Sanders, J. L., Newman, A. B., Odden, M. C., & Sciences, M. (2017). A modified healthy aging index and its association with mortality: The National Health and Nutrition Examination Survey, 1999. *Journals of Gerontology Series A: Biomedical Sciences*, *72*(10), 1437–1444.

Xia, S., Zhang, X., Zheng, S., Khanabdali, R., Kalionis, B., Wu, J., Wan, W., & Tai, X. (2016). An Update on Inflamm-Aging: Mechanisms, Prevention, and Treatment. *J Immunol Res*, *2016*, 8426874. https://doi.org/10.1155/2016/8426874

Xie, Y. (2020). *Knitr: A general-purpose package for dynamic report generation in r* [Manual].

Xu, M., Tchkonia, T., Ding, H., Ogrodnik, M., Lubbers, E. R., Pirtskhalava, T., White, T. A., Johnson, K. O., Stout, M. B., Mezera, V., Giorgadze, N., Jensen, M. D., LeBrasseur, N. K., & Kirkland, J. L. (2015). JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. *Proceedings of the National Academy of Sciences*, *112*(46), E6301–E6310. https://doi.org/10.1073/pnas.1515386112

Yaiw, K.-C., Ovchinnikova, O., Taher, C., Mohammad, A.-A., Davoudi, B., Shlyakhto, E., Rotar, O., Konradi, A., Wilhelmi, V., Rahbar, A., Butler, L., Assinger, A., & Söderberg-Nauclér, C. (2013). High prevalence of human cytomegalovirus in carotid atherosclerotic plaques obtained from Russian patients undergoing carotid endarterectomy. *Herpesviridae*, *4*(1), 3. https://doi.org/10.1186/2042-4280-4-3

Yang, Y., & Kozloski, M. (2011). Sex differences in age trajectories of physiological dysregulation: Inflammation, metabolic syndrome,

and allostatic load. *Journals of Gerontology Series A: Biomedical Sciences Medical Sciences*, *66*(5), 493–500.

Zhu, H. (2020). *kableExtra: Construct complex table with 'kable' and pipe syntax* [Manual].

Nederlandse samenvatting (Summary in Dutch)

Een goed werkend immuunsysteem is essentieel om gezond ouder te kunnen worden. Het is onmisbaar voor de bescherming tegen ziektekiemen, en voor het opruimen van dode cellen en afvalstoffen in het lichaam. Ook speelt het een belangrijke rol in het repareren van schade aan weefsels in het lichaam. Het immuunsysteem bestaat uit veel verschillende eiwitten, cellen en moleculen die op een zeer complexe manier met elkaar samenwerken en communiceren. Al deze verschillende immunologische cellen en stoffen houden elkaar in evenwicht. Bij een juiste balans worden schadelijke ziektekiemen snel geneutraliseerd, terwijl het eigen, gezonde weefsel niet wordt beschadigd. Als de balans tussen al deze cellen en stoffen van het immuunsysteem verstoord raakt, kan het zo zijn dat het immuunsysteem zijn werk slechter gaat vervullen, en mogelijk zelfs schade aanricht aan het eigen lichaam. Het slechter functioneren van het immuunsysteem komt vaker voor op oudere leeftijd, maar niet bij iedereen. De vraag is waarom dit bij sommige mensen wel gebeurt, en bij anderen niet. We kunnen een inzicht krijgen in het immuunsysteem doordat we verschillenden elementen en aspecten van dit systeem kunnen onderzoeken en meten. Zo kunnen we een aantal 'immunologische biomarkers' definiëren, die een beeld geven van het functioneren van het immuunsysteem.

Dit proefschrift is gericht op het identificeren van immunologische biomarkers van *frailty*. *Frailty* is een breed begrip en komt vaker voor naarmate men ouder wordt. Iemand is *frail* als diegene kwetsbaar is, een relatief hoge kans op ongunstige gebeurtenissen heeft zoals een val of een delirium, en minder goed van zulke ongunstige gebeurtenissen kan herstellen (Clegg et al., 2013; Kenneth Rockwood & Howlett, 2019; Walston et al., 2006). Er worden in dit proefschrift nieuwe inzichten gepresenteerd in hoe gemeten waardes van immunologische biomarkers veranderen met de (leef)tijd in een populatie van verouderende personen, en hoe deze veranderingen mogelijk samenhangen met de kans om *frail* te worden. Ook wordt onderzocht of de communicatie tussen immunologische eiwitten (cytokines) en immuuncellen is verstoord. Dit doen we door de reacties te meten die plaatsvinden binnenin immuuncellen wanneer ze met deze cytokines worden gestimuleerd, via een specifiek activatiemechanisme (*'JAK/STAT pathway'*).

In **Hoofdstuk 2** beschrijven we de personen van wie er data worden gebruikt in dit proefschrift. Het zijn mensen uit de omgeving van Doetinchem die vanaf 1987 elke vijf jaar deel hebben genomen aan de 'Doetinchem Cohort Studie,' waarin veel van informatie over hun gezondheid is verzameld. Met allerlei onderzoeken zijn hun lichamelijk, cognitief en psychisch functioneren gemeten, en er zijn telkens meerdere bloedmonsters afgenomen. Verder ontwikkelen we in dit hoofdstuk een manier om *frailty* in een getal uit te drukken, door een *'frailty index score'* (K. Rockwood et al., 2011) samen te stellen in het Doetinchem Cohort. Deze score bestaat uit 36 verschillende kenmerken van ongezondheid welke bij de proefpersonen waren gemeten, of met een vragenlijst uitgevraagd. Analyses lieten zien dat hogere scores voor *frailty* gecorreleerd waren met kalenderleeftijd, zoals verwacht. Ook hingen hogere scores samen met een hoger sterfterisico, ongeacht de kalenderleeftijd. Dit komt overeen met wat gevonden is in andere studies waarin ook een *frailty index* was ontwikkeld en gebruikt (Collerton et al., 2012; K. Rockwood et al., 2011; Schoufour et al., 2017; Searle et al., 2008).

In het bloed van 289 personen uit de Doetinchem cohort studie bepaalden we concentraties van leukocyten (witte bloedcellen). Leukocyten kunnen op basis van hun afstamming vanuit de stamcellen worden onderverdeeld in *myeloïde* en *lymfoïde* cellen. In **Hoofdstuk 2** zagen we in mannen en vrouwen met een hoge score voor *frailty* een groter aantal myeloïde cellen. Ook zagen we in dezelfde groep een chronisch hogere concentratie van de ontstekingsmarker CRP (*'C-reactive protein'*) over een tijdsspanne van 20 jaar. Dit suggereert dat chronische inflammatie (ontsteking) een rol speelt in het proces waardoor mensen *frail* worden op oudere leeftijd.

Een grote groep verschillende immuuncellen vormt samen het *'cellulaire immuunprofiel'*. Bij het meer in detail bestuderen hiervan vielen verschillen tussen mannen en vrouwen op: vrouwen hadden over het algemeen meer B – en T cellen, maar minder monocyten in het bloed (**Hoofdstuk 3**). Ook zagen we dat mannen en vrouwen een verschillend profiel hadden dat met *frailty* samenhing (**Hoofdstuk 3**). Dit profiel liet zien dat verschillen in aantallen immuuncellen mogelijk een grotere rol spelen bij vrouwen dan bij mannen in de processen die tot *frailty* leiden. Kenmerkend in dit profiel waren de hogere aantallen myeloïde cellen, zoals neutrofielen en monocyten. Deze myeloïde cellen hadden een sterkere associatie met *frailty* dan T- of B cellen; de associatie met *frailty* was ook sterker dan die tussen *frailty* en cellen waarvan bekend is dat de concentraties veranderen met hogere leeftijd, zoals lagere aantallen 'naïeve' $CD8^+$ T cellen maar juist hogere aantallen 'inflammatie regulerende' T cellen en hogere aantallen 'geheugen' T cellen, dat wil zeggen 'rijpe' cellen die zich niet verder differentiëren of delen. Deze veranderingen in het aantal T cellen met leeftijd hoeven dus niet te betekenen dat iemand *frail* is.

In **Hoofdstuk 4** worden verouderingspatronen in de algemene populatie in Nederland geanalyseerd, waarvoor we de Doetinchem studie als representatief hebben genomen. Dit deden we door een *'healthy aging index'* samen te stellen. De *healthy aging index* heeft minder variabelen dan de uitgebreide

frailty index, maar toch nog genoeg om een schatting te maken van de algehele lichamelijke gezondheid. Deze score kon gemeten worden over de tijd in de proefpersonen van het Doetinchem cohort. Hierdoor konden we met verschillende algoritmes verouderingspatronen in de algehele bevolking onderzoeken over meerdere jaren. Zoals verwacht, zagen we een afname in deze score, en dus tekenen van achteruitgang van lichamelijke functies, relatief vroeg in de levensloop, vanaf ongeveer 45 jaar. In mannen identificeerden we twee verschillende verouderingstrajecten, één waarin de veroudering min of meer 'geleidelijk' plaatsvond, en een kleinere groep waarin deze veroudering relatief snel plaatsvond. In vrouwen werd één patroon ontdekt, dat grotendeels samenviel met dat van de snel verouderende mannen. Belangrijke factoren welke een risico vormden om tot de snel verouderende groep te horen, waren een hoge *'Body Mass Index'* (BMI) en een lage sociaal economische status.

Het is bekend dat een chronische cytomegalovirus (CMV) infectie van invloed is op de aantallen immuuncellen in het bloed (met name de geheugen T cellen). Omdat het immuunsysteem constant zijn best moet doen om deze virusinfectie onder controle te houden, wordt vaak aangenomen dat een CMV infectie zorgt voor snellere veroudering. In **Hoofdstuk 5** deden we onderzoek naar de rol van dit virus, en we lieten hier zien dat besmet raken met CMV niet sterk samenhing met *frailty*. Aangezien we bloedmonsters van de proefpersonen hadden tot 30 jaar terug in de tijd, konden we ook bepalen wanneer iemand geïnfecteerd was geraakt, en zo ook hoe lang iemand geïnfecteerd was geweest met het virus. We zagen geen sterke relatie tussen de duur van infectie en *frailty*. Verder zagen we inderdaad veel hogere aantallen rijpe T cellen in personen die geïnfecteerd waren met CMV, zoals verwacht (Wertheimer et al., 2014), maar opnieuw maakte het voor deze celaantallen niet veel uit of iemand heel lang (>25 jaar) of juist kortgeleden geïnfecteerd was. De theorie dat cumulatieve blootstelling van het lichaam aan een chronische CMV infectie voor toenemende 'stress' zorgt op het immuunsysteem en daarmee voor snellere

algemene veroudering, konden daarom niet met onze resultaten ondersteund worden.

In meerdere artikelen is beschreven dat er in oudere mannen en vrouwen vaak een chronische, laaggradige inflammatie optreedt, wat mogelijk komt door een minder adequate immuunrespons (Daniel Baylis et al., 2013; Claudio Franceschi et al., 2006). Bij het gedetailleerd bestuderen van indicatoren van chronische inflammatie (inflammatoire biomarkers), en hun verandering in concentratie over de tijd, zagen we dat markers gerelateerd aan IFNγ en aan bloedplaatjesactivatie sterk gerelateerd waren aan veranderingen van de meeste andere markers, en dus mogelijk een centrale plaats innemen in een inflammatoir biomarkerprofiel (**Hoofdstuk 6**). We zagen ook meerdere duidelijke verschillen tussen mannen vrouwen in de biomarker concentraties en veranderingen daarin. Deze verschillen werden minder duidelijk na de leeftijd van ongeveer 60 jaar. Mogelijk spelen hier hormonale veranderingen rond de menopauze een rol. De inflammatoire markers geassocieerd met *frailty* waren de markers gerelateerd aan de IL-6 signalerings *'pathway'*. In vrouwen zagen we ook dat met de leeftijd toenemende concentraties van sCD14, een marker van monocyten activatie, gerelateerd waren aan *frailty*. De meeste associaties verdwenen na het corrigeren voor BMI; dit laat zien dat BMI en overgewicht mogelijk belangrijke factoren kunnen zijn die het immuunmarker profiel dat gerelateerd is aan *frailty* mede bepalen.

Als laatste hebben we nog onderzocht of de functie van immuuncellen anders is in kwetsbare, 'frail,' ouderen vergeleken met gezonde ouderen (**Hoofdstuk 7**). Hier zagen we dat de immuuncellen van *frail* ouderen de signalen van specifieke immuunmarkers ('cytokines') minder goed verwerken en minder goede respons (reactie) hebben op die signalen. Dit zagen we in B cellen, in T cellen, en in monocyten. De responsen die we hier gemeten hadden waren 'pSTAT' responsen (pSTAT1, pSTAT3, en pSTAT5). Dit kan een teken zijn dat laaggradige inflammatie het immuunsysteem 'uitput,' met verminderde signalering via de zogenoemde 'JAK/STAT pathway' tot gevolg,

wat mogelijk dus leidt tot slechtere immuunresponsen in *frail* personen. Van belang is verder ook dat verminderde IL10- geïnduceerde pSTAT3 responsen werden gezien in zowel mannen die *frail* waren als in mannen met chronisch hogere levels van een ontstekingsmarker (CRP). Aangezien IL10 een immuunregulerende werking heeft, zou het dus kunnen zijn dat *frail* mannen minder goed immuunreacties kunnen 'dempen.'

Conclusie

Het is een cruciale vraag waarom sommige mensen gezond oud worden en anderen niet. Om deze vraag te beantwoorden is het zeer belangrijk te weten wat de rol van het immuunsysteem hierin is. Dit proefschrift is erop gericht om immunologische biomarkers te vinden welke gerelateerd zijn aan veroudering en, met name, *frailty* op oudere leeftijd. Specifiek keken we naar hoe verschillende immunologische biomarkers met elkaar in verband staan, hoe de concentraties van deze biomarkers veranderen over de tijd, en hoe deze veranderingen zijn gerelateerd aan klinisch relevante tekenen van immuunveroudering. Verder hebben we onderzocht welke immunologische mechanismen belangrijk zijn in het ontwikkelen van een sluimerende chronische ontsteking.

We vonden inderdaad een 'immuunprofiel' gerelateerd aan *frailty*. We zagen tekenen van ontregeling van immuuncellen uit de myeloïde cellijn. Ook zagen we verschillen tussen mannen en vrouwen; vrouwen lieten meer en ook sterkere associaties zien tussen cellulaire en moleculaire immuunmarker en *frailty*. Dit laat zien dat het van belang is om mannen en vrouwen apart te onderzoeken bij het bestuderen van veroudering. Gebaseerd op onze resultaten lijkt een chronische CMV infectie minder dan verwacht *frailty* te beïnvloeden. Ten slotte zagen we dat het verwerken van signalen van cytokines mogelijk verstoord is in immuuncellen van *frail* ouderen. Mogelijk kunnen deze resultaten houvast bieden om in de toekomst een behandeling te

vinden tegen veroudering van het immuunsysteem. Een belangrijke hypothese van ons was dat laaggradige inflammatie vooraf gaat aan het ontstaan van *frailty*. Hoewel we geen definitieve conclusies kunnen trekken, vonden we inderdaad aanwijzingen welke deze hypothese ondersteunen.

De resultaten uit dit proefschrift laten zien hoe het immuunsysteem betrokken is in het zeer complexe, multifactoriële verouderingsproces. Nieuwe immuunmarkers zijn hier ontdekt, welke verschillen tussen mannen en vrouwen en een aanvulling zijn op de al bekende klinische markers gerelateerd aan veroudering. We hopen daarom dat dit proefschrift een opstap is voor vervolgstudies om nieuwe technieken toe te passen in cohorten waarin proefpersonen langdurig gevolgd zijn, om zo uiteindelijk steeds beter te kunnen voorspellen welke personen gezond verouderen en welke niet.

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