Mechanisms of Ageing and Development 133 (2012) 456–466

Contents lists available at SciVerse ScienceDirect

Mechanisms of Ageing and Development

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

Frailty and the role of inflammation, immunosenescence and cellular ageing in the very old: Cross-sectional findings from the Newcastle 85+ Study

Joanna Collerton [a,*], Carmen Martin-Ruiz [a], Karen Davies [a], Catharien M. Hilkens [b], John Isaacs [b,c], Claire Kolenda [a], Craig Parker [a], Michael Dunn [b], Michael Catt [a], Carol Jagger [a], Thomas von Zglinicki [a], Thomas B.L. Kirkwood [a]

[a] Institute for Ageing and Health, Newcastle University, Campus for Ageing and Vitality, Newcastle upon Tyne NE4 5PL, United Kingdom
[b] Institute of Cellular Medicine, Newcastle University, Framlington Place, Newcastle upon Tyne NE2 4HH, United Kingdom
[c] Musculoskeletal Directorate, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne NE7 7DN, United Kingdom

ARTICLE INFO

Article history:
Received 27 March 2012
Received in revised form 4 May 2012
Accepted 16 May 2012
Available online 1 June 2012

Keywords:
Frailty
Inflammation
Immunosenescence
Telomere
Oxidative stress

ABSTRACT

Age-related frailty is an increasing societal challenge with growing emphasis on identifying its underlying pathophysiology and prospects for intervention. We report findings from the first comprehensive study of frailty and biomarkers of inflammation, immunosenescence and cellular ageing in the very old. Using cross-sectional data from the Newcastle 85+ Study (n = 845, aged 85), frailty was operationalized by the Fried and Rockwood models and biomarker associations explored using regression analysis. We confirmed the importance of inflammatory markers (IL-6, TNF-alpha, CRP, neutrophils) in frailty in the very old, previously established only in younger–old populations. Limited evidence was found for immunosenescence in frailty; although total lymphocyte count was inversely related, no association was found with the immune risk profile and the inverse associations observed with memory/naïve CD8 T and B cell ratios were in the opposite direction to that expected. We found no association with frailty in the very old for CMV sero-positivity, telomere length, markers of oxidative stress or DNA damage and repair. The Fried and Rockwood frailty models measure different albeit overlapping concepts yet biomarker associations were generally consistent between models. Difficulties in operationalizing the Fried model, due to high levels of co-morbidity, limit its utility in the very old.

1. Introduction

Age-related frailty is an increasing challenge for societies worldwide, with growing emphasis on identifying its underlying pathophysiology and prospects for intervention. It is generally agreed that frailty is characterised by increased vulnerability to stress due to decline in homeostatic reserve secondary to dysregulation in multiple inter-related systems (Bortz, 2002; Fried et al., 2001; Lipsitz, 2002; Walston et al., 2006). This vulnerability results in an increased risk of adverse health outcomes including disability, hospitalisation, institutionalisation and death (Bandeen-Roche et al., 2006; Fried et al., 2001; Kulminski et al., 2007; Mitnitski et al., 2005; Rockwood et al., 2011; Romero-Ortuno et al., 2011). Despite concerted efforts (Bergman et al., 2007; Rodríguez-Mañas et al., 2012; Walston et al., 2006), there is as yet no universally accepted definition of frailty or agreed method for its diagnosis. Existing approaches differ widely in how frailty is conceptualised and defined (Abellan van Kan et al., 2008; Hogan et al., 2003; Sternberg et al., 2011), with the approaches of Fried and Rockwood currently leading the field. Fried views frailty as a clinical syndrome – a cluster of specific symptoms and signs including weight loss, exhaustion, low physical activity, muscle weakness and slow walking speed (Fried et al., 2001). Rockwood considers frailty as a cumulative index of health deficits; it is regarded as a clinical state variable and proposed to act as an indicator of biological rather than chronological age (Rockwood and Mitnitski, 2007; Searle et al., 2008). The individual deficits can include diseases, symptoms and signs, function tests and laboratory tests. Provided enough deficits are included in the index, their exact nature seems unimportant (Rockwood et al., 2006).

Abbreviations: FFS, Fried frailty status; RFI, Rockwood frailty index.

* Corresponding author. Tel.: +44 191 2481100; fax: +44 191 2481101.
E-mail addresses: joanna.collerton@ncl.ac.uk (J. Collerton), carmen.martin-ruiz@ncl.ac.uk (C. Martin-Ruiz), karen.davies@ncl.ac.uk (K. Davies), catharien.hilkens@ncl.ac.uk (C.M. Hilkens), john.isaacs@ncl.ac.uk (J. Isaacs), claire.kolenda@ncl.ac.uk (C. Kolenda), craig.parker@ncl.ac.uk (C. Parker), michael.dunn3@ncl.ac.uk (M. Dunn), michael.catt@ncl.ac.uk (M. Catt), carol.jagger@ncl.ac.uk (C. Jagger), t.vonzglinicki@ncl.ac.uk (T. von Zglinicki), tom.kirkwood@ncl.ac.uk (Thomas B.L. Kirkwood).

0047-6374/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.mad.2012.05.005

Knowledge of the pathophysiological mechanisms underlying frailty remains limited. It is unclear to what extent frailty has its own specific causative mechanisms, as distinct from the more general deterioration of cellular and physiological functions which comprise the ageing process, which might be amenable to targeted interventions. Whilst Fried frailty has been associated with dysregulation in several systems, (Bandeen-Roche et al., 2009; Barzilay et al., 2007; Blaum et al., 2009; Cappola et al., 2009; Leng et al., 2007; Shardell et al., 2009; Travison et al., 2011; Walston et al., 2002), there are few investigations of underlying mechanisms for the Rockwood frailty index. Studies exploring the role of cellular and molecular mechanisms of ageing are scarce for either model, and biomarker investigations using both frailty models within the same sample are rare.

People aged 85 years and over are now the most rapidly expanding age group in the population with current numbers predicted to double over the next 20 years (United Nations, 2002). Biomarker associations with health outcomes may differ between very old and younger old populations; for example telomere length predicts mortality in younger old (Cawthon et al., 2003) but not in very old populations (Bischoff et al., 2006; Houben et al., 2011; Martin-Ruiz et al., 2005). The applicability of the Rockwood and Fried frailty measures in very old people has not been addressed.

The Newcastle 85+ Study, a population-based study of a large representative cohort of 85 year olds, collected comprehensive measures of health across multiple biological, clinical and psychosocial domains (Collerton et al., 2009). We examine the applicability and inter-relations of the Fried and Rockwood frailty measures within this cohort, and their associations with a range of biomarkers of inflammation, immunosenescence and cellular ageing.

2. Methods

2.1. Study population

The methodology for the Newcastle 85+ Study has been reported (Collerton et al., 2007, 2009; Davies et al., 2010). In brief, members of the 1921 birth cohort living in Newcastle or North Tyneside (North-East England) were recruited at around age 85 through general practice patient lists. People living in institutions and those with cognitive impairment were included. At baseline the Newcastle 85+ cohort was socio-demographically representative of the local population and of England and Wales (Collerton et al., 2009; Office for National Statistics, 2004). The research complied with the requirements of the Declaration of Helsinki. Ethical approval was obtained from the Newcastle and North Tyneside 1 Research Ethics Committee (reference number 06/Q0905/2); written informed consent was obtained from participants and where people lacked capacity to consent, for example because of dementia, a formal written opinion was sought from a relative or carer.

2.2. Data sources

A multidimensional health assessment was carried out in the participant's usual residence by a research nurse. Data on pre-existing diseases and prescribed medication were obtained from general practice medical records.

2.3. Biomarker analysis

Biomarkers were measured from a blood sample drawn between 7 am and 10.30 am following an 8 h overnight fast and delivered to the laboratory for initial processing within one hour of draw. Blood samples were collected within 6 months of participant assessment with the exception of cytomegalovirus (CMV) status where the timeframe was within 18 months. The following biomarkers were analysed according to previously reported methodology (Martin-Ruiz et al., 2011).

i. Inflammatory markers: basal and lipopolysaccharide (Invivogen Ultrapure LPS) stimulated production of pro-inflammatory cytokines interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-alpha) by peripheral blood mononuclear cells (PBMCs); C-reactive protein (CRP) measured by high sensitivity assay; albumin; total white blood cell count and counts of neutrophils, monocytes, eosinophils and basophils.
ii. Immunosenescence markers: lymphocyte count and ratios of CD4/CD8 T cells, memory/naïve CD4 and CD8 T cells and memory/naïve B cells. The immune risk profile was defined as a CD4/CD8 T cell ratio of less than one (Wikby et al., 1998, 2008).
iii. Cytomegalovirus serology: CMV IgG concentration was measured using a commercial assay.
iv. Cellular ageing markers: PBMC telomere length; ionized radiation-induced DNA damage and repair; and markers of oxidative stress. Isoprostanes iPF2 alpha-III and iPF2 alpha-VI, markers of lipid peroxidation, were analysed by liquid chromatography–mass spectrometry.

Further details of biomarker methodology are reported in Supplementary Methods (Appendix A).

2.4. Frailty measures

Fried frailty status (FFS), robust, pre-frail or frail, was derived using an approximation of the Cardiovascular Health Study methodology (Fried et al., 2001). In line with the stipulated methodology, participants with stroke, Parkinson's Disease, a mini-mental state examination score of less than 18, or taking drugs for dementia, Parkinson's Disease or depression were excluded on the basis that they might score as frail as a result of that disease alone. The Rockwood frailty index (RFI), a continuous variable with theoretical range 0–1, was computed from 40 potential deficits following the methodology reported by Searle et al. (2008). For further details of the FFS and RFI methodology see Supplementary Methods (Appendix A).

2.5. Other data reported

A count of 18 chronic diseases was calculated (Collerton et al., 2009); the full list of diseases is shown in the footnote to Table 1. Cognitive status was assessed using the standardised mini-mental state examination (Molloy and Standish, 1997). Body mass index was calculated from measured weight and height (derived from demi-span). Ethnicity, place of residence, years of education, smoking status (current smoker, ex-smoker, never), and difficulties with activities of daily living were obtained by self-report. A disability score (maximum 17) was calculated from the total number of activities of daily living performed with difficulty, or requiring an aid or appliance or personal help (Collerton et al., 2009).

2.6. Data analysis

Differences between RFI samples with and without FFS and between RFI and FFS samples with and without blood tests were examined using a chi-square test for nominal data and a Mann–Whitney *U* test for ordinal and non-parametric continuous data. Sex differences in frailty were examined by ordinal logistic regression for FFS and a Mann–Whitney *U* test for RFI. Due to non-normal distributions, continuously distributed biomarkers were categorised into quartiles based on their distribution in the full sample. Differences in the proportion classified as frail, pre-frail or robust (FFS) and in RFI by biomarker category were examined using a Mann–Whitney *U* test for CMV status and immune risk profile and a Kruskal–Wallis test for ordinal biomarker categories. The correlation between RFI and continuous biomarker level was assessed using Spearman's rank correlation. Ordinal logistic regression models were fitted with FFS as the dependent variable and categorised biomarker as the independent variable. The middle two biomarker quartiles were combined and used as the reference category to facilitate identification of potential relationships at the top and bottom of the distribution. The odds ratios obtained represent the odds of being in a more frail Fried category i.e. pre-frail rather than robust or frail rather than pre-frail. The test of parallel lines was used to check that the proportional odds assumption was satisfied. For RFI, linear regression models were fitted with RFI, square root transformed to give adequate model fit, as the dependent variable and categorised biomarker as independent variable. The unstandardized regression coefficient represents the difference between the mean square root frailty indices for the biomarker category and the reference category. Models were run unadjusted (Model 1), adjusted for sex only (Model 2) and adjusted for a range of co-variates (Model 3). Co-variates for both frailty models were sex, years of education (0–9, 10–11, 12+ years), smoking status and the use of oral corticosteroids, non-steroidal anti-inflammatory drugs, anti-infection or immune modulating drugs. For FFS a count of chronic diseases (categorised as tertiles) was added and for RFI body mass index. Co-variates used in the derivation of frailty status were excluded. p Values less than 0.05 were taken as statistically significant. We did not apply a formal statistical correction for multiple comparisons as many of the markers examined are known to exhibit strong correlations with each other reflecting common biological mechanisms. In this situation, tests for associations between individual biomarkers and frailty were not independent and therefore a Bonferroni correction for multiple comparisons would have been over-conservative. Data analysis was conducted using SPSS Version 19 (IBM Inc., Chicago, USA).

3. Results

3.1. Sample selection

Details of sample selection are shown in Supplementary Fig. A1 (Appendix A). Data from both health assessment and review of

Table 1
Key socio-demographic and health parameters – reported separately for sample with Rockwood frailty index (RFI) and RFI samples with and without Fried frailty status (FFS)[a]. Comparison of RFI samples with/without FFS.

	Sample with RFI (n = 811)	Sample with RFI and FFS (n = 552)	Sample with RFI but without FFS (n = 259)	p Value
Female	61.7 (500)	60.1 (332)	64.9 (168)	0.197[b]
White ethnicity	99.6 (808)	99.6 (550)	99.6 (258)	0.959[b]
Place of residence				<0.001[b]
Standard housing	77.6 (629)	84.6 (467)	62.5 (162)	
Sheltered housing	12.8 (104)	13.0 (72)	12.4 (32)	
Resident in care home	9.5 (77)	2.2 (12)	25.1 (65)	
Years of education				0.428[c]
12+	12.4 (99)	12.5 (69)	12.1 (30)	
10–11	23.4 (187)	22.2 (122)	26.2 (65)	
0–9	64.2 (512)	65.3 (359)	61.7 (153)	
Smoking status				0.804[b]
Never smoker	35.4 (286)	34.6 (191)	37.0 (95)	
Ex-smoker	59.0 (477)	59.6 (329)	57.6 (148)	
Current smoker	5.7 (46)	5.8 (32)	5.4 (14)	
Body mass index (kg/m^2)				0.573[c]
<18.50	6.6 (47)	7.2 (38)	4.8 (9)	
18.50–24.99	51.4 (368)	50.4 (266)	54.3 (102)	
25.00–29.99	32.5 (233)	31.3 (165)	36.2 (68)	
≥30.00	9.5 (68)	11.2 (59)	4.8 (9)	
Cognitive function (mini-mental state examination score)				<0.001[c]
Normal (26–30)	73.9 (582)	82.4 (449)	54.7 (133)	
Mildly impaired (22–25)	15.0 (118)	14.5 (79)	16.0 (39)	
Moderately impaired (18–21)	4.9 (39)	3.1 (17)	9.1 (22)	
Severely impaired (0–17)	6.2 (49)	0.0 (0)	20.2 (49)	
Chronic disease count, median (IQR)[d]	5 (4–6)	4 (3–6)	5 (4–7)	<0001[c]
Disability score, median (IQR)[e]	3 (1–7)	2 (0–5)	7 (2–12)	<0001[c]
Rockwood frailty index, median (IQR)	0.20 (0.13–0.28)	0.18 (0.11–0.24)	0.26 (0.19–0.38)	<0001[c]

[a] Data are % (n) except where specified; denominators vary due to missing values.
[b] Chi-square test for no significant difference between RFI samples with and without FFS.
[c] Mann–Whitney U test for no significant difference between RFI samples with and without FFS.
[d] Eighteen diseases: hypertension, ischaemic heart disease, cerebrovascular disease, peripheral vascular disease, heart failure, atrial flutter or fibrillation, arthritis, osteoporosis, chronic obstructive pulmonary disease or asthma, other respiratory disease, diabetes mellitus, hypothyroidism or hyperthyroidism, cancer diagnosed within past 5 years (excluding non-melanoma skin cancer), eye disease, dementia, Parkinson's Disease, renal impairment and anaemia.
[e] Number of activities of daily living performed with difficulty or requiring an aid, appliance or personal help (maximum score 17).

general practice records were available for 845 participants (Collerton et al., 2009). Of these, RFI could be calculated for 811 participants (96.0%); the remaining 34 had more than the allowed 8 missing values. FFS could be assigned for 552 participants (65.3%); applying the Fried exclusion criteria resulted in loss of 252 participants and an additional 41 could not be assigned due to missing values on individual Fried criteria. RFI was available for all participants with FFS. Biomarker results were available, depending on assay, for 612–771 of those with RFI and 423–526 of those with FFS (Supplementary Table A1, Appendix A).

3.2. Sample characteristics

Table 1 shows key socio-demographic and health data for the sample with RFI available and the RFI samples with and without FFS. Supplementary Table S1 (Appendix A) gives corresponding biomarker distributions. In comparison to those without FFS, those with FFS were less likely to be resident in a care home, less likely to have impaired cognitive function, had a lower disease count and lower RFI, were less disabled, and had lower CRP, neutrophil count and stimulated TNF-alpha and higher albumin and memory/naïve CD8 T cell ratio. For those with FFS, a comparison of those who did and did not have a blood sample taken revealed no significant differences other than educational status, those who had blood taken being more educated than those who did not (p = 0.036). A similar comparison for the RFI sample showed that those who had blood taken were less likely to be resident in a care home (p = 0.001), less likely to be cognitively impaired (p = 0.012) and had lower disability (p = 0.001) and RFI scores (p = 0.030).

3.3. Frailty

3.3.1. Frailty by Fried and Rockwood measures

Using the Fried measure, 21.6% (119/552) of participants were classified as frail, 60.3% (333/552) as pre-frail and 18.1% (100/552) as robust (Table 2). Women were more likely to be frail than men:

Table 2
Frailty by Rockwood and Fried models, by gender.

	Men	Women	All	p Value
Rockwood frailty index, median (IQR)[a]	0.18 (0.12–0.24)	0.21 (0.14–0.29)	0.20 (0.13–0.28)	<0.001[c]
Fried frailty status, % (n)[b]				<0.001[d]
Robust	24.1 (53)	14.2 (47)	18.1 (100)	
Pre-frail	63.6 (140)	58.1 (193)	60.3 (333)	
Frail	12.3 (27)	27.7 (92)	21.6 (119)	

[a] Sample size n = 811.
[b] Sample size n = 552.
[c] Mann–Whitney U test for no gender difference.
[d] Ordinal logistic regression for no gender difference.

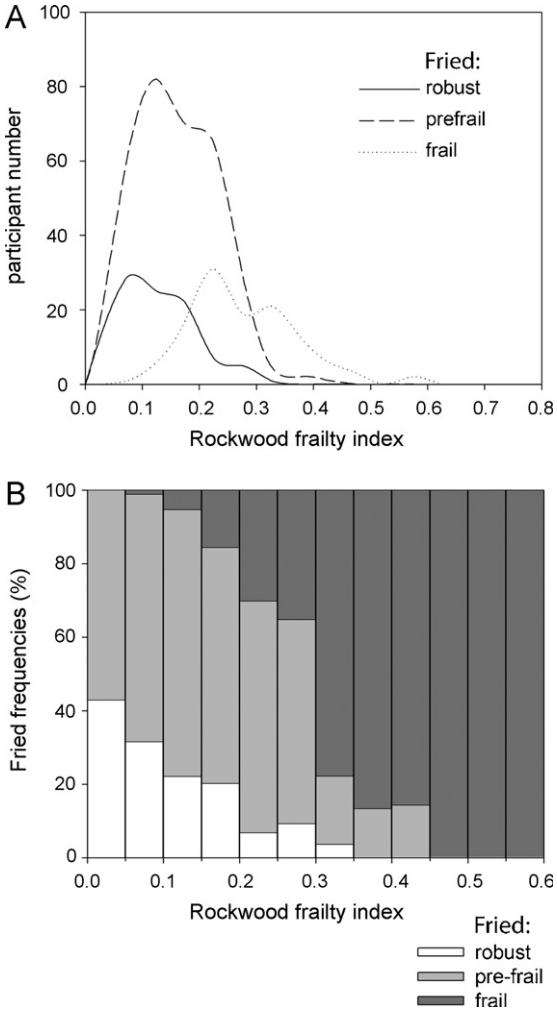

Fig. 1. Relationship between Rockwood frailty index (RFI) and Fried frailty status (FFS). Panel A shows the smoothed RFI distribution for each FFS category. In Panel B the RFI for the full FFS sample has been split into bins of width 0.05 with the percentage in each FFS category shown for each bin.

odds ratio (95% confidence interval, CI) for women to men 2.27 (1.61–3.23); $p < 0.001$. RFI had a gamma distribution with median (interquartile range, IQR) 0.20 (0.13–0.28), mean (standard deviation) 0.22 (0.12) and 99th centile 0.59; it was higher in women than men ($p < 0.001$).

3.3.2. Relationship between Fried and Rockwood frailty

RFI increased across Fried categories: median (IQR) for robust (0.12, 0.08–0.17), pre-frail (0.16, 0.11–0.22) and frail (0.27, 0.21–0.34); $p < 0.001$. Fig. 1 illustrates the relationship between FFS and RFI. Panel A shows the smoothed RFI distribution for each Fried category. Within each Fried category there was a wide distribution of RFI scores with considerable overlap in distributions particularly between the robust and pre-frail groups; RFI scores between 0.08 and 0.34 could be found in all three Fried categories. In Panel B the RFI for the full FFS sample has been split into bins of width 0.05 with the percentage in each Fried category shown for each bin. As RFI increased, the proportion classified as Fried robust decreased whilst the proportion classified as frail increased; the pre-frail proportion stayed similar up to a frailty index of 0.30 then decreased.

3.4. Relationship between frailty and biomarkers

3.4.1. Fried frailty status (FFS) and biomarkers

Unadjusted ordinal logistic regression revealed significant associations with FFS for seven biomarkers: basal IL-6, basal TNF-alpha, CRP, neutrophil count, albumin, lymphocyte count, and memory/naïve CD8 T cell ratio (Table 3, Model 1). Compared to the combined middle (referent) quartiles, being in the bottom quartile for basal IL-6 or TNF-alpha was associated with a lower risk of frailty; odds ratios (95% CI) 0.48 (0.31–0.74), $p = 0.001$ and 0.59 (0.38–0.90), $p = 0.016$ respectively. A greater risk of frailty was associated with high levels of CRP and neutrophils (odds ratios (95% CI) 1.82 (1.19–2.80), $p = 0.006$ and 1.65 (1.06–2.56), $p = 0.027$ respectively). Low albumin levels were associated with a greater risk of frailty; odds ratio (95% CI) 2.16 (1.39–3.37), $p = 0.001$. For lymphocytes and memory/naïve CD8 T cell ratio, high levels were associated with a lower risk of frailty; odds ratios (95% CI) 0.59 (0.39–0.90), $p = 0.015$ and 0.63 (0.41–0.97), $p = 0.036$ respectively. When albumin, basal

Table 3
Fried frailty status and biomarkers – odds ratio (95% confidence intervals) of being in a frailer category (pre-frail versus robust or frail versus pre-frail) by categorised biomarker.[a]

Biomarker		Model 1 odds ratio (95% confidence interval)	Model 1 *p* value	Model 2 odds ratio (95% confidence interval)	Model 2 *p* value	Model 3 odds ratio (95% confidence interval)	Model 3 *p* value
CMV							
CMV positive		0.94 (0.57–1.55)	0.796	0.90 (0.54–1.49)	0.680	0.74 (0.42–1.28)	0.276
CMV negative		*Reference*		*Reference*		*Reference*	
Inflammation							
IL-6, basal (pg/ml)	≤4.15	0.48 (0.31–0.74)	0.001	0.47 (0.30–0.73)	0.001	0.50 (0.31–0.79)	0.003
	4.16–150.02	*Reference*		*Reference*		*Reference*	
	≥150.03	0.92 (0.60–1.43)	0.719	0.96 (0.62–1.50)	0.873	0.91 (0.57–1.47)	0.710
TNF-alpha, basal (pg/ml)	≤1.58	0.59 (0.38–0.90)	0.016	0.60 (0.39–0.92)	0.021	0.62 (0.39–0.98)	0.041
	1.59–8.92	*Reference*		*Reference*		*Reference*	
	≥8.93	0.90 (0.58–1.40)	0.644	0.97 (0.62–1.51)	0.897	0.88 (0.54–1.43)	0.605
IL-6, post stimulation (pg/ml)	≤10543.27	0.94 (0.61–1.44)	0.774	0.82 (0.53–1.26)	0.372	0.89 (0.56–1.41)	0.612
	10543.28–31366.09	*Reference*		*Reference*		*Reference*	
	≥31366.10	0.84 (0.54–1.30)	0.426	0.77 (0.49–1.20)	0.244	0.66 (0.41–1.06)	0.087
TNF-alpha, post stimulation(pg/ml)	≤213.35	0.75 (0.49–1.15)	0.187	0.71 (0.46–1.08)	0.111	0.81 (0.51–1.27)	0.357
	213.36–905.78	*Reference*		*Reference*		*Reference*	
	≥905.79	0.94 (0.60–1.47)	0.770	0.95 (0.60–1.49)	0.814	0.92 (0.57–1.50)	0.737
CRP (mg/l)	≤1.20	1.36 (0.91–2.05)	0.137	1.36 (0.90–2.04)	0.143	1.41 (0.90–2.19)	0.131
	1.21–6.00	*Reference*		*Reference*		*Reference*	
	≥6.01	1.82 (1.19–2.80)	0.006	1.97 (1.28–3.03)	0.002	1.78 (1.12–2.85)	0.016
Albumin (g/l)	≤38	2.16 (1.39–3.37)	0.001	2.12 (1.36–3.30)	0.001	1.79 (1.11–2.89)	0.017
	39–42	*Reference*		*Reference*		*Reference*	
	≥43	0.83 (0.53–1.30)	0.425	0.85 (0.54–1.33)	0.481	0.89 (0.55–1.43)	0.630

Table 3 (*Continued*)

Biomarker		Model 1 odds ratio (95% confidence interval)	Model 1 *p* value	Model 2 odds ratio (95% confidence interval)	Model 2 *p* value	Model 3 odds ratio (95% confidence interval)	Model 3 *p* value
White blood cells ($\times 10^9$/l)	≤5.40	1.24 (0.82–1.88)	0.309	1.18 (0.77–1.79)	0.448	1.18 (0.76–1.84)	0.464
	5.41–7.60	*Reference*		*Reference*		*Reference*	
	≥7.61	1.45 (0.93–2.26)	0.103	1.48 (0.94–2.31)	0.087	1.23 (0.77–1.98)	0.391
Neutrophils ($\times 10^9$/l)	≤2.87	0.87 (0.58–1.33)	0.527	0.84 (0.55–1.28)	0.412	0.85 (0.54–1.32)	0.464
	2.88–4.55	*Reference*		*Reference*		*Reference*	
	≥4.56	1.65 (1.06–2.56)	0.027	1.68 (1.08–2.62)	0.021	1.47 (0.92–2.36)	0.109
Monocytes ($\times 10^9$/l)	≤0.44	1.07 (0.70–1.62)	0.768	1.04 (0.68–1.58)	0.858	0.98 (0.63–1.55)	0.946
	0.45–0.66	*Reference*		*Reference*		*Reference*	
	≥0.67	0.94 (0.61–1.46)	0.790	1.05 (0.67–1.63)	0.833	0.95 (0.60–1.50)	0.827
Eosinophils ($\times 10^9$/l)	≤0.13	0.79 (0.52–1.21)	0.277	0.74 (0.48–1.13)	0.161	0.72 (0.46–1.13)	0.152
	0.14–0.31	*Reference*		*Reference*		*Reference*	
	≥0.32	0.85 (0.55–1.32)	0.476	0.92 (0.60–1.42)	0.706	0.85 (0.53–1.34)	0.475
Basophils ($\times 10^9$/l)	≤0.020	1.37 (0.92–2.04)	0.121	1.41 (0.94–2.09)	0.093	1.36 (0.89–2.08)	0.150
	0.021–0.050	*Reference*		*Reference*		*Reference*	
	≥0.051	1.57 (1.00–2.46)	0.052	1.55 (0.98–2.44)	0.059	1.55 (0.96–2.51)	0.076
Immunosenescence							
Lymphocytes ($\times 10^9$/l)	≤1.43	1.09 (0.71–1.67)	0.705	1.16 (0.75–1.78)	0.503	1.25 (0.79–1.98)	0.344
	1.44–2.23	*Reference*		*Reference*		*Reference*	
	≥2.24	0.59 (0.39–0.90)	0.015	0.55 (0.36–0.84)	0.006	0.57 (0.37–0.90)	0.016
Immune risk profile (CD4/CD8 <1)		0.92 (0.58–1.46)	0.723	1.11 (0.69–1.77)	0.670	0.98 (0.58–1.66)	0.949
CD4/CD8 ≥1		*Reference*		*Reference*		*Reference*	
CD4/CD8 T cell ratio	≤1.19	0.84 (0.54–1.29)	0.426	0.96 (0.62–1.49)	0.856	0.88 (0.55–1.41)	0.588
	1.20–3.52	*Reference*		*Reference*		*Reference*	
	≥3.53	1.11 (0.72–1.72)	0.645	1.05 (0.68–1.63)	0.819	1.01 (0.63–1.62)	0.965
Memory/naïve CD4 T cell ratio	≤0.12	0.80 (0.52–1.22)	0.297	0.77 (0.50–1.18)	0.233	0.84 (0.53–1.32)	0.442
	0.13–0.62	*Reference*		*Reference*		*Reference*	
	≥0.63	0.80 (0.52–1.23)	0.309	0.89 (0.58–1.38)	0.609	1.01 (0.63–1.62)	0.971
Memory/naïve CD8 T cell ratio	≤0.37	0.97 (0.62–1.51)	0.891	0.99 (0.64–1.54)	0.967	1.12 (0.70–1.81)	0.637
	0.38–2.75	*Reference*		*Reference*		*Reference*	
	≥2.76	0.63 (0.41–0.97)	0.036	0.70 (0.46–1.08)	0.111	0.61 (0.38–0.98)	0.043
Memory/naïve B cell ratio	≤0.18	1.42 (0.90–2.24)	0.127	1.36 (0.86–2.14)	0.185	1.15 (0.70–1.87)	0.582
	0.19–0.69	*Reference*		*Reference*		*Reference*	
	≥0.70	1.29 (0.84–1.99)	0.244	1.33 (0.86–2.05)	0.199	1.29 (0.81–2.05)	0.281
Cellular ageing							
Telomere length (bp)	≤3345.55	0.80 (0.51–1.24)	0.314	0.76 (0.48–1.18)	0.215	0.76 (0.47–1.24)	0.278
	3345.56–4237.39	*Reference*		*Reference*		*Reference*	
	≥4237.40	1.10 (0.71–1.70)	0.663	1.19 (0.77–1.84)	0.429	1.14 (0.71–1.81)	0.597
DNA damage (%)	≤31.26	1.20 (0.79–1.83)	0.402	1.18 (0.77–1.80)	0.448	1.24 (0.79–1.95)	0.358
	31.27–65.08	*Reference*		*Reference*		*Reference*	
	≥65.09	1.01 (0.67–1.53)	0.959	1.03 (0.68–1.57)	0.888	0.96 (0.61–1.51)	0.854
DNA repair (%)	≤24.10	1.14 (0.74–1.74)	0.556	1.23 (0.80–1.88)	0.351	1.17 (0.74–1.84)	0.512
	24.11–65.05	*Reference*		*Reference*		*Reference*	
	≥65.06	1.13 (0.74–1.71)	0.575	1.14 (0.75–1.72)	0.547	1.36 (0.87–2.14)	0.179
Isoprostanes (ng/ml)							
iPF2alpha-III	≤0.54	0.89 (0.58–1.37)	0.606	0.90 (0.59–1.38)	0.631	0.88 (0.56–1.38)	0.568
	0.55–2.83	*Reference*		*Reference*		*Reference*	
	≥2.84	0.97 (0.63–1.48)	0.874	0.96 (0.63–1.48)	0.865	1.08 (0.68–1.73)	0.748
iPF2alpha-VI	≤2.71	1.04 (0.68–1.60)	0.838	1.07 (0.69–1.63)	0.772	1.05 (0.67–1.65)	0.829
	2.72–11.43	*Reference*		*Reference*		*Reference*	
	≥11.44	1.15 (0.74–1.77)	0.535	1.22 (0.79–1.88)	0.373	1.26 (0.78–2.02)	0.343

[a] Ordinal logistic regression models were fitted with Fried status as the dependent variable and categorised biomarker as the independent variable. Continuously distributed biomarkers were quartiled and the middle two quartiles were combined and used as the reference category. Model 1 is unadjusted; Model 2 is adjusted for sex only; and Model 3 is adjusted for sex, education, smoking status, number of chronic diseases, and use of oral corticosteroids, non-steroidal anti-inflammatory, anti-infection or immune modulating drugs.

IL-6, basal TNF-alpha and CRP were entered into a model together, low albumin remained significant with an attenuated odds ratio 1.91 (95% CI 1.19–3.09), $p = 0.008$. When models were fully adjusted, the findings for basal IL-6 and TNF-alpha, CRP, albumin, lymphocytes and memory/naïve CD8 T cell ratio remained significant; effect sizes were broadly similar except for albumin where the odds ratio dropped by 0.37 (Table 3, Model 3). Supplementary Table A2 (Appendix A) shows the proportion classified as frail, pre-frail and robust within each biomarker category.

3.4.2. Rockwood frailty index and biomarkers

Using Spearman's rank correlation, a significant association with RFI was found for eight biomarkers: basal IL-6, basal TNF-alpha, CRP, albumin, white cell count, neutrophil count, lymphocyte count and memory/naïve B cell ratio (Table 4). Positive correlations were seen for basal IL-6, basal TNF-alpha, CRP, white cell count and neutrophil count, and negative for albumin, lymphocyte count and memory/naïve B cell ratio. The largest effect sizes were seen for albumin, CRP and neutrophil count yet these biomarkers explained only 7.3%, 4.7%, and 3.4% respectively of RFI variance.

Unadjusted linear regression models for RFI (square root transformed) showed significant associations with six biomarkers: basal IL-6, basal TNF-alpha, CRP, albumin, neutrophil count and memory/naïve B cell ratio (Table 5, Model 1). Compared to participants in the combined middle (referent) quartiles, participants with low basal IL-6 and TNF-alpha were significantly less frail (lower mean square root frailty index), whilst those with high CRP and neutrophils and low albumin and memory/naïve B cell ratio were significantly more frail. Absolute differences in mean

Table 4
Rockwood frailty index and biomarkers (continuous variables) – Spearman's rank correlation.

Biomarker	Spearman's rho	*p* Value
Inflammation		
IL-6, basal (pg/ml)	0.086	0.023
TNF-alpha, basal (pg/ml)	0.101	0.007
IL-6, post stimulation (pg/ml)	0.046	0.229
TNF-alpha, post stimulation(pg/ml)	0.051	0.181
CRP (mg/l)	0.217	<0.001
Albumin (g/l)	−0.270	<0.001
White blood cells ($\times 10^9$/l)	0.103	0.005
Neutrophils ($\times 10^9$/l)	0.185	<0.001
Monocytes ($\times 10^9$/l)	0.020	0.579
Eosinophils ($\times 10^9$/l)	−0.018	0.633
Basophils ($\times 10^9$/l)	−0.064	0.080
Immunosenescence		
Lymphocytes ($\times 10^9$/l)	−0.076	0.039
CD4/CD8 T cell ratio	0.034	0.374
Memory/naïve CD4 T cell ratio	−0.017	0.644
Memory/naïve CD8 T cell ratio	−0.073	0.054
Memory/naïve B cell ratio	−0.089	0.020
Cellular ageing		
Telomere length (bp)	−0.008	0.824
DNA damage (%)	−0.028	0.452
DNA repair (%)	−0.056	0.126
Isoprostanes (ng/ml)		
iPF2alpha-III	−0.041	0.278
iPF2alpha-VI	0.020	0.588

Table 5
Rockwood frailty index (RFI) and biomarkers – regression coefficients (unstandardized) for square root transformed RFI by categorised biomarker.[a]

Biomarker		Model 1 unstandardized regression coefficient (95% confidence interval)	Model 1 *p* value	Model 2 unstandardized r egression coefficient (95% confidence interval)	Model 2 *p* value	Model 3 unstandardized regression coefficient (95% confidence interval)	Model 3 *p* value
CMV							
CMV positive		0.012 (−0.014 to 0.037)	0.370	0.010 (−0.015 to 0.036)	0.433	−0.002 (−0.026 to 0.022)	0.879
CMV negative		*Reference*		*Reference*		*Reference*	
Inflammation							
IL-6, basal (pg/ml)	≤4.15	−0.030 (−0.052 to −0.007)	0.010	−0.031 (−0.053 to −0.008)	0.007	−0.022 (−0.043 to −0.001)	0.041
	4.16–150.02	*Reference*		*Reference*		*Reference*	
	≥150.03	0.003 (−0.019 to 0.026)	0.761	0.005 (−0.017 to 0.027)	0.649	0.016 (−0.005 to 0.037)	0.143
TNF-alpha, basal (pg/ml)	≤1.58	−0.025 (−0.047 to −0.002)	0.033	−0.024 (−0.046 to −0.002)	0.035	−0.021 (−0.043 to 0.000)	0.048
	1.59–8.92	*Reference*		*Reference*		*Reference*	
	≥8.93	0.001 (−0.022 to 0.024)	0.922	0.003 (−0.019 to 0.026)	0.770	0.011 (−0.010 to 0.032)	0.299
IL-6, post stimulation	≤10543.27	−0.011 (−0.033 to 0.012)	0.358	−0.016 (−0.038 to 0.007)	0.169	−0.012 (−0.034 to 0.009)	0.261
(pg/ml)	10543.28–31366.09	*Reference*		*Reference*		*Reference*	
	≥31366.10	0.008 (−0.015 to 0.031)	0.494	0.008 (−0.015 to 0.030)	0.491	0.004 (−0.017 to 0.025)	0.727
TNF-alpha, post	≤213.35	−0.014 (−0.036 to 0.009)	0.231	−0.017 (−0.040 to 0.005)	0.133	−0.017 (−0.039 to 0.004)	0.112
stimulation (pg/ml)	213.36–905.78	*Reference*		*Reference*		*Reference*	
	≥905.79	0.008 (−0.015 to 0.030)	0.499	0.009 (−0.014 to 0.031)	0.446	0.005 (−0.017 to 0.026)	0.658
CRP (mg/l)	≤1.20	−0.020 (−0.041 to 0.001)	0.062	−0.019 (−0.040 to 0.001)	0.067	−0.011 (−0.031 to 0.009)	0.297
	1.21–6.00	*Reference*		*Reference*		*Reference*	
	≥6.01	0.049 (0.028–0.070)	<0.001	0.050 (0.030–0.071)	<0.001	0.035 (0.014–0.055)	0.001
Albumin (g/l)	≤38	0.092 (0.072–0.112)	<0.001	0.090 (0.070–0.110)	<0.001	0.052 (0.031–0.072)	<0.001
	39–42	*Reference*		*Reference*		*Reference*	
	≥43	−0.008 (−0.031 to 0.014)	0.472	−0.006 (−0.028 to 0.016)	0.592	−0.010 (−0.031 to 0.011)	0.355
White blood cells ($\times 10^9$/l)	≤5.40	−0.008 (−0.029 to 0.013)	0.468	−0.010 (−0.031 to 0.011)	0.358	−0.006 (−0.026 to 0.015)	0.580
	5.41–7.60	*Reference*		*Reference*		*Reference*	
	≥7.61	0.022 (−0.001 to 0.044)	0.056	0.021 (−0.001 to 0.043)	0.059	0.016 (−0.004 to 0.037)	0.122
Neutrophils ($\times 10^9$/l)	≤2.87	−0.018 (−0.039 to 0.004)	0.106	−0.019 (−0.040 to 0.002)	0.077	−0.017 (−0.037 to 0.003)	0.101
	2.88–4.55	*Reference*		*Reference*		*Reference*	
	≥4.56	0.036 (0.014–0.057)	0.001	0.036 (0.015–0.058)	0.001	0.027 (0.006–0.048)	0.010
Monocytes ($\times 10^9$/l)	≤0.44	-0.003 (-0.024 to 0.019)	0.788	-0.003 (-0.024 to 0.018)	0.794	-0.009 (-0.029 to 0.012)	0.415
	0.45–0.66	*Reference*		*Reference*		*Reference*	
	≥0.67	−0.008 (−0.030 to 0.014)	0.466	−0.002 (−0.024 to 0.020)	0.842	−0.011 (−0.032 to 0.010)	0.317
Eosinophils ($\times 10^9$/l)	≤0.13	0.004 (−0.017 to 0.026)	0.706	0.001 (−0.020 to 0.022)	0.930	−0.006 (−0.027 to 0.014)	0.539
	0.14–0.31	*Reference*		*Reference*		*Reference*	
	≥0.32	0.003 (−0.019 to 0.025)	0.780	0.006 (−0.016 to 0.028)	0.591	0.001 (−0.020 to 0.021)	0.927
Basophils ($\times 10^9$/l)	≤0.020	0.009 (−0.011 to 0.029)	0.363	0.009 (−0.011 to 0.029)	0.368	0.010 (−0.009 to 0.029)	0.315
	0.021–0.050	*Reference*		*Reference*		*Reference*	
	≥0.051	−0.013 (−0.036 to 0.011)	0.281	−0.014 (−0.037 to 0.009)	0.229	−0.006 (−0.028 to 0.016)	0.585
Immunosenescence							
Lymphocytes ($\times 10^9$/l)	≤1.43	0.009 (−0.013 to 0.030)	0.428	0.012 (−0.009 to 0.033)	0.258	0.012 (−0.009 to 0.032)	0.268
	1.44–2.23	*Reference*		*Reference*		*Reference*	
	≥2.24	−0.021 (−0.043 to 0.001)	0.057	−0.024 (−0.045 to −0.002)	0.031	−0.018 (−0.038 to 0.003)	0.086

Table 5 (*Continued*)

Biomarker		Model 1 unstandardized regression coefficient (95% confidence interval)	Model 1 *p* value	Model 2 unstandardized r egression coefficient (95% confidence interval)	Model 2 *p* value	Model 3 unstandardized regression coefficient (95% confidence interval)	Model 3 *p* value
Immune risk profile (CD4/CD8 T cell ratio <1)		−0.006 (−0.030 to 0.019)	0.648	0.001 (−0.023 to 0.026)	0.907	−0.004 (−0.028 to 0.020)	0.734
CD4/CD8 T cell ratio ≥1		*Reference*		*Reference*		*Reference*	
CD4/CD8 T cell ratio	≤1.19	0.005 (−0.018 to 0.027)	0.687	0.010 (−0.013 to 0.032)	0.391	−0.001 (−0.022 to 0.021)	0.963
	1.20–3.52	*Reference*		*Reference*		*Reference*	
	≥3.53	0.014 (−0.009 to 0.037)	0.226	0.010 (−0.012 to 0.033)	0.364	0.010 (−0.012 to 0.031)	0.377
Memory/naïve	≤0.12	−0.007 (−0.029 to 0.016)	0.567	−0.008 (−0.030 to 0.014)	0.473	−0.007 (−0.028 to 0.015)	0.548
CD4 T cell ratio	0.13–0.62	*Reference*		*Reference*		*Reference*	
	≥0.63	−0.005 (−0.028 to 0.017)	0.637	0.000 (−0.023 to 0.022)	0.976	−0.002 (−0.023 to 0.020)	0.880
Memory/naïve	≤0.37	0.011 (−0.012 to 0.033)	0.356	0.012 (−0.010 to 0.035)	0.277	0.019 (−0.002 to 0.041)	0.078
CD8 T cell ratio	0.38–2.75	*Reference*		*Reference*		*Reference*	
	≥2.76	−0.019 (−0.042 to 0.003)	0.097	−0.014 (−0.036 to 0.009)	0.232	−0.010 (−0.031 to 0.012)	0.379
Memory/naïve	≤0.18	0.029 (0.006 to 0.052)	0.012	0.028 (0.005 to 0.050)	0.016	0.032 (0.011 to 0.053)	0.003
B cell ratio	0.19–0.69	*Reference*		*Reference*		*Reference*	
	≥0.70	0.008 (−0.015 to 0.030)	0.488	0.009 (−0.013 to 0.031)	0.437	−0.006 (−0.027 to 0.016)	0.593
Cellular ageing							
Telomere length (bp)	≤3345.55	0.004 (−0.018 to 0.026)	0.718	0.001 (−0.022 to 0.023)	0.965	0.000 (−0.021 to 0.022)	0.965
	3345.56–4237.39	*Reference*		*Reference*		*Reference*	
	≥4237.40	−0.005 (−0.028 to 0.017)	0.645	−0.003 (−0.025 to 0.020)	0.814	0.008 (−0.013 to 0.029)	0.455
DNA damage (%)	≤31.26	0.002 (−0.020 to 0.024)	0.877	0.001 (−0.021 to 0.023)	0.916	0.009 (−0.012 to 0.029)	0.403
	31.27–65.08	*Reference*		*Reference*		*Reference*	
	≥65.09	−0.008 (−0.030 to 0.014)	0.454	−0.009 (−0.031 to 0.013)	0.413	−0.006 (−0.027 to 0.015)	0.564
DNA repair (%)	≤24.10	0.002 (−0.020 to 0.024)	0.830	0.004 (−0.017 to 0.026)	0.695	0.007 (−0.013 to 0.028)	0.478
	24.11–65.05	*Reference*		*Reference*		*Reference*	
	≥65.06	−0.010 (−0.032 to 0.012)	0.355	−0.009 (−0.031 to 0.012)	0.398	−0.005 (−0.025 to 0.016)	0.665
Isoprostanes (ng/ml)							
iPF2alpha-III	≤0.54	0.002 (−0.020 to 0.024)	0.843	0.004 (−0.018 to 0.026)	0.733	0.003 (−0.018 to 0.024)	0.747
	0.55–2.83	*Reference*		*Reference*		*Reference*	
	≥2.84	−0.012 (−0.034 to 0.010)	0.296	−0.011 (−0.033 to 0.011)	0.342	−0.002 (−0.023 to 0.019)	0.840
iPF2alpha-VI	≤2.71	−0.005 (−0.027 to 0.018)	0.676	−0.002 (−0.024 to 0.020)	0.876	0.000 (−0.021 to 0.021)	0.991
	2.72–11.43	*Reference*		*Reference*		*Reference*	
	≥11.44	0.007 (−0.015 to 0.029)	0.529	0.011 (−0.011 to 0.033)	0.317	0.015 (−0.006 to 0.037)	0.152

[a] Linear regression models were fitted with Rockwood frailty index (square root transformed) as the dependent variable and categorised biomarker as independent variable. Continuously distributed biomarkers were quartiled and the middle two quartiles were combined and used as the reference category. Model 1 is unadjusted; Model 2 is adjusted for sex only; and Model 3 is adjusted for sex, education, smoking status, body mass index, and use of oral corticosteroids, non-steroidal anti-inflammatory, anti-infection or immune modulating drugs.

square root RFI ranged from 0.092 for albumin to 0.025 for basal TNF-alpha. When albumin, basal IL-6, basal TNF-alpha and CRP were entered into a model together, low albumin remained significant ($p < 0.001$) with an attenuated regression coefficient (0.082, 95% CI 0.060–0.104). When models were adjusted for sex (Table 5, Model 2), participants with high lymphocytes were significantly less frail, a finding which was borderline significant in the unadjusted model. When models were fully adjusted the findings for basal IL-6, basal TNF-alpha, CRP, albumin, neutrophil count and memory/naïve B cell ratio remained significant with attenuated regression coefficients, with the exception of memory/naïve B cell ratio where the coefficient increased (Table 5, Model 3). The median RFI in each biomarker category is shown in Supplementary Table A3 (Appendix A). For biomarkers significant in the regression analysis, the absolute differences in median RFI compared to the referent category were 0.08 for albumin; 0.04 for CRP; 0.03 for neutrophils; 0.02 for basal TNF-alpha, lymphocytes and memory/naïve B cell ratio; and 0.01 for basal IL-6.

Linear regression models were repeated using the smaller healthier RFI sample with FFS available (data not shown). For unadjusted models, the findings for CRP, neutrophils, albumin and memory/naïve B cell ratio remained significant (p values < 0.001, <0.001, 0.017 and 0.011 respectively). For white cell count the borderline significant result in the full RFI sample result became significant (p value 0.026). These findings retained significance, with attenuated regression coefficients, in fully adjusted models.

3.4.3. Consistency of biomarker associations across frailty measures

Findings were generally consistent across both frailty measures when using the full sample for each ($n = 811$ for RFI, $n = 552$ for FFS). Exceptions were white blood cells and memory/naïve B cell ratio where findings were confined to RFI, and memory/naïve CD8 T cell ratio where associations found with FFS were of borderline significance for RFI and confined to correlation analysis.

4. Discussion

4.1. Overview of findings

This is the most comprehensive assessment to date of biomarker associations with objectively determined frailty in a very old population. We confirmed the importance of inflammatory markers in frailty in a very old population, previously established only in the younger old. CRP, IL-6, TNF-alpha and neutrophil count showed positive associations with both frailty measures and albumin inverse associations. Limited evidence was found to support the role of immunosenescence. Although total lymphocyte count was inversely related to both frailty measures, no relationships were found with the immune risk profile and the inverse relationships observed with memory/naïve CD8 T and B cell ratios were in the opposite direction to that expected. No frailty associations were found with CMV sero-positivity or the cellular ageing markers. Although the two frailty models measure different albeit overlapping concepts (Cigolle et al., 2009; Rockwood et al., 2007), findings were generally consistent across both measures.

Effect sizes for the Fried frailty measure were relatively large and fairly consistent across markers. Being in the outer biomarker quartiles was associated with approximately double or half the risk of being in a frailer Fried category, compared to the middle referent quartiles. For the Rockwood index, absolute differences in RFI

scores between the outer and referent quartiles ranged from 0.01 (IL-6) to 0.08 (albumin). This represents a change in the sample median RFI of 5–40%, which equates to 0.4–3.2 heath deficits out of a total of 40. With the exception of some white cell parameters, associations were independent of a range of confounders.

4.2. Inflammation and frailty

The only previous investigation of inflammatory markers and Rockwood frailty is that of Hubbard et al. (2009) in 110 people aged 75+. CRP, IL-6 and TNF-alpha were positively correlated with RFI and albumin inversely; no correlation was found for total white blood cell count whilst white cell sub-types were not addressed.

A number of studies have explored inflammation and Fried frailty, although several excluded men (Fried et al., 2009; Leng et al., 2007, 2009a,b; Reiner et al., 2009; Schmaltz et al., 2005), or had relatively small samples (Hubbard et al., 2009; Leng et al., 2002, 2004a,b, 2011b; Serviddio et al., 2009; Wu et al., 2009). IL-6 is consistently associated with Fried frailty in both cross-sectional (Bandeen-Roche et al., 2009; Fried et al., 2009; Hubbard et al., 2009; Leng et al., 2002, 2004a,b, 2007, 2011b; Matheï et al., 2011; Schmaltz et al., 2005) and prospective studies (Reiner et al., 2009), whilst evidence for CRP and TNF-alpha is mixed. For CRP, associations with prevalent and incident frailty were found in the Cardiovascular Health Study (Barzilay et al., 2007; Walston et al., 2002), and Hubbard et al. (2009) and Wu et al. (2009) reported association with prevalent frailty. However, no association with incident frailty was found in the Women's Health Initiative-Observational Study (Reiner et al., 2009) or with prevalent frailty in the InCHIANTI study (Bandeen-Roche et al., 2009). For TNF-alpha, Serviddio et al. (2009) and Hubbard et al. (2009) found an association with prevalent frailty, whilst no association was seen in the INCHIANTI study (Bandeen-Roche et al., 2009) or by Leng et al. (2004b).

With the exception of Leng et al. (2004b), previous studies measured circulating levels of IL-6 and TNF-alpha whereas we measured basal and stimulated production by PBMCs. Whilst IL-6 and TNF-alpha are not produced exclusively by PBMCs, basal PBMC production is likely to be closely related to circulating levels. In contrast, stimulated cytokine levels reflect the ability of PBMCs to mount an immune response. The only previous investigation of frailty and basal and stimulated PBMC production of IL-6 and TNF-alpha is that by Leng et al. (2004b) ($n = 22$). No frailty association was found for basal levels of either cytokine whilst stimulated IL-6 was associated but not stimulated TNF-alpha. We found no association between frailty and stimulated PBMC production of IL-6 or TNF-alpha.

With respect to cellular components of the inflammatory response, we found an association with neutrophils for both frailty measures, for white blood cells with Rockwood only, and no association for monocytes, eosinophils and basophils for either measure. The Women's Health and Ageing Studies reported associations between Fried frailty and white cells (Leng et al., 2007, 2009a), neutrophils and monocytes (Leng et al., 2009b) although the finding for neutrophils and monocytes was confined to a disabled subset. In contrast, Hubbard et al. (2009) found no association with white cell count and Fried frailty, although sub-types were not explored. The effect of the total white cell count can be difficult to interpret as it is the sum of its component sub-types; of note the frailty relationships we found for neutrophils and lymphocytes were in opposite directions.

Our finding of an inverse association between albumin and frailty is in line with previous studies of the Fried (Hubbard et al., 2009; Le Couteur et al., 2010; Walston et al., 2002; Wu et al., 2009) and Rockwood models (Hubbard et al., 2009). The association persisted, with some attenuation, after adjustment for inflammatory markers indicating the importance of albumin as a marker of more than one system; low albumin is associated with inflammation, malnutrition and disease states.

The importance of inflammation in frailty is reinforced by the consistency in findings across different frailty models (Hubbard et al., 2008, 2009; Almeida et al., 2012; Puts et al., 2005), together with reports of the significance of the burden of inflammatory disease (Chang et al., 2010, 2012).

4.3. CMV and frailty

We found no association between CMV sero-positivity and either frailty model. Chronic CMV infection is a driver of T cell immunosenescence and chronic pro-inflammatory states (Derhovanessian et al., 2009; Mogensen and Paludan, 2001; Pawelec et al., 2009). No previous studies have examined CMV status and Rockwood frailty; findings for the Fried model are conflicting. Positive association was found for CMV sero-positivity and prevalent frailty (Schmaltz et al., 2005), and for high anti-CMV IgG titres and incident frailty (Wang et al., 2010) in the Women's Health and Ageing Studies. Conversely, the Belfrail study in men and women aged 80+ reported an inverse association between CMV sero-positivity and prevalent frailty and no association with anti-CMV IgG titres (Matheï et al., 2011).

Heterogeneity in the CMV sero-positive older population has been suggested as a possible explanation for these inconsistencies, with potential differences between study populations in the proportion of sero-positive participants with persistent chronic infection (Leng, 2011). Positive CMV serology cannot distinguish between persistent and resolved infection (Leng et al., 2011a). Although it has been suggested that anti-CMV IgG titre levels might reflect the cumulative frequency of viral reactivation (Wang et al., 2010), no definitive evidence exists. A survival effect in the very old has also been proposed with those susceptible to the adverse effects of CMV under-represented in the sample due to death at an earlier age (Matheï et al., 2011).

4.4. Immunosenescence and frailty

We found that total lymphocyte count, a crude marker of immune status, was inversely associated with both Fried and Rockwood frailty. No previous studies have explored links between Rockwood frailty and lymphocyte count. Three previous studies of the Fried model found no association, although these had relatively small samples (De Fanis et al., 2008; Semba et al., 2005) and/or excluded men (Leng et al., 2009b; Semba et al., 2005).

Alteration in T lymphocyte subsets is a key marker of immunosenescence. A decrease in the number and functionality of naïve cells and an increase in the number of memory cells but showing poor functionality are important. Lifetime antigenic burden is an important driver with CMV a key pathogen (Derhovanessian et al., 2009; Larbi et al., 2008; Pawelec et al., 2009). A CD4/CD8 T cell ratio less than one, termed the immune risk profile (IRP), was a key immunosenescence marker in the Swedish OCTO and NONA studies of people aged over 85 (Ferguson et al., 1995; Wikby et al., 1998, 2005). No previous studies have investigated immunosenescence and Rockwood frailty. Five studies have explored immunosenescence in Fried frailty. A study in the Women's Health and Ageing cohort ($n = 127$) reported an association with frailty and low CD4/CD8 T cell ratio and high CD8, low CD4, high CD8+CD28−, low CD8+CD28+, high CD4+CD28− and low CD4+CD28+ T cells (Semba et al., 2005). De Fanis et al. (2008) ($n = 26$) found an association with frailty and low CD4 and high CD8 T cell counts. In the Multicentre Aids Cohort, low CD4 T cell counts in 245 HIV infected men were independently predictive of a frailty-related phenotype (Desquilbet et al., 2007). In the NONA

study lower B cell diversity was associated with 'frailty'; however frailty was defined as not medicated, institutionalised or demented rather than being objectively determined (Gibson et al., 2009). In contrast, we found no association with the immune risk profile for either frailty model and the inverse relationships observed for the memory/naïve CD8 T cell and B cell ratios were in the opposite direction to that anticipated. The recent observation that currently used immune phenotyping markers may not adequately differentiate between senescent and fit memory T cells (Prlic et al., 2012) may offer a partial explanation.

4.5. Cellular markers of ageing and frailty

We found no association between frailty and oxidative stress, PBMC telomere length or DNA damage and repair capacity. No previous studies have explored the role of oxidative stress in Rockwood frailty, whilst three report an association with Fried frailty. Wu et al. (2009) ($n = 90$) and Serviddio et al. (2009) ($n = 62$) found frailty associations with 8 hydroxy-2 deoxyguanosine, and oxidised glutathione plus markers of lipid peroxidation respectively. The InCHIANTI study ($n = 827$) used an indirect measure of oxidative stress, the antioxidant Vitamin E, and found an inverse association with frailty (Ble et al., 2006). Only one study investigated the relationship between telomere length and frailty; Woo et al. (2008) found no correlation between peripheral blood telomere length and the Rockwood frailty index in a sample of 2000 Hong-Kong Chinese. As yet, there are no published human population-based studies reporting on the associations between DNA damage/repair markers and ageing phenotypes including frailty.

4.6. The importance of studying dysregulation across multiple inter-related systems

We have considered the relationship between frailty and biomarkers of a limited range of systems/processes, with each marker considered in isolation. Other systems are also implicated in frailty with dysregulation across multiple systems a key mechanism (Fried et al., 2009; Gruenewald et al., 2009; Sanders et al., 2011; Szanton et al., 2009). Complex interactions occur between markers and between systems (De Martinis et al., 2006; Derhovanessian et al., 2009; Fulop et al., 2010; Hummel and Abecassis, 2002; Kregel and Zhang, 2007; Larbi et al., 2007; Pawelec et al., 2009; Prosch et al., 1999; von Zglinicki and Martin-Ruiz, 2005). Further research into the pathophysiology of frailty will require sophisticated analytical techniques, such as those used in systems biology (Kirkwood, 2008, 2011), to integrate findings from the extensive range of physiological systems and cellular and molecular processes potentially involved, and their complex interactions.

4.7. Applicability of the Rockwood and Fried frailty models in the very old

We were able to operationalize RFI in 96% of our sample. FFS could not be determined in over one third of our sample, due largely to the requirement to exclude participants with certain pre-existing conditions on the basis that these might cause a participant to be scored as frail due to the effects of that disease alone. This was compounded by non-completion of performance tests in a population with considerable multi-morbidity (Collerton et al., 2009). This significantly limits the utility of the Fried model in very old people.

4.8. Strengths and limitations

The strengths of this study are its size and the range of biomarkers addressed. A further important aspect is the population-based nature of the sample which included those living in institutions and the cognitively impaired. Domiciliary assessment facilitated the participation of those unable or unwilling to travel for clinic based assessment. Our analyses were adjusted for a range of potential confounders, including disease burden for the Fried measure, and recruitment of a single year birth cohort removed the confounding effect of age.

Some limitations deserve comment. Our data is so far only cross-sectional; we will investigate the predictive effects of biomarkers on frailty transitions as longitudinal data becomes available. Whilst the recruitment rate was high for study of this kind and the study sample was socio-demographically representative, it is possible that non-respondents were frailer than those who participated. For the Fried model this is compounded by the stipulated exclusion criteria; hence our Fried frailty prevalence will under-estimate the scale of the problem. As in many other investigations (Avila-Funes et al., 2008; Cawthon et al., 2007; Cigolle et al., 2009; Ensrud et al., 2007; Santos-Eggimann et al., 2009; Wilhelm-Leen et al., 2010), our FFS operationalization was an approximation of the Cardiovascular Health Study methodology as the exact variables were not collected. However our frailty prevalence was identical to that reported in the Cardiovascular Health Study for this age group (Fried et al., 2001). CMV status was determined at the 18 month follow-up assessment rather than at baseline for 80% of the sample; however we consider rates of sero-conversion between the ages of 85 and 86.5 years to be very low. As stated in the methods, we could not reliably perform a formal statistical correction for multiple testing. We are nevertheless confident that our principal findings concerning the role of inflammatory markers in frailty in the very old are robust being also in strong agreement with previous studies in younger populations. However, p values around 0.05 should be treated with appropriate caution.

4.9. Conclusions

This large population-based study of 85 year olds confirms the importance of inflammatory markers in frailty in the very old, previously established only in the younger old. Limited evidence was found for the role of immunosenescence in frailty in this age group, and no evidence for CMV sero-positivity, telomere length, oxidative stress or DNA damage and repair. Difficulties in operationalizing the Fried model in the very old limit its utility in this age group.

Funding

This work was supported by the UK Medical Research Council and the Biotechnology and UK Biological Sciences Research Council (G0500997), and the UK Medical Research Council and Unilever Discover Colworth (G0601333). The funders had no role in the study design; in the collection, analysis and interpretation of data; in the writing of the paper; and in the decision to submit the paper for publication.

Conflict of interest statement

None declared.

Acknowledgements

Thanks are especially due to the 85 year olds of Newcastle and North Tyneside for the generous donation of their time and personal information to make the study possible. In addition we thank: the research nurses (Brenda Balderson, Sally Barker, Julie Burrows, June Edwards, Julie Ferguson, Gill Hedley, Joan Hughes, Judith Hunt and Victoria Raynor); biomarker technicians (Sam

Jameson, and Anna Tang); data manager (Pauline Potts); project statistician (Andrew Kingston); project secretary (Lucy Farfort); Wendy den Elzen (for assistance with preparation of biomarker datasets); and NHS North of Tyne (Newcastle Primary Care Trust) and local general practices.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mad.2012.05.005.

References

Abellan van Kan, G., Rolland, Y., Bergman, H., Morley, J.E., Kritchevsky, S.B., Vellas, B., 2008. The I.A.N.A. Task Force on frailty assessment of older people in clinical practice. The Journal of Nutrition, Health & Aging 12, 29–37.

Almeida, O.P., Norman, P.E., van Bockxmeer, F.M., Hankey, G.J., Flicker, L., 2012. CRP 1846G>A polymorphism increases risk of frailty. Maturitas 71, 261–266.

Avila-Funes, J.A., Helmer, C., Amieva, H., Barberger-Gateau, P., Le Goff, M., Ritchie, K., Portet, F., Carriere, I., Tavernier, B., Gutierrez-Robledo, L.M., Dartigues, J.-F., 2008. Frailty among community-dwelling elderly people in France: the three-city study. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 63, 1089–1096.

Bandeen-Roche, K., Walston, J.D., Huang, Y., Semba, R.D., Ferrucci, L., 2009. Measuring systemic inflammatory regulation in older adults: evidence and utility. Rejuvenation Research 12, 403–410.

Bandeen-Roche, K., Xue, Q.-L., Ferrucci, L., Walston, J., Guralnik, J.M., Chaves, P., Zeger, S.L., Fried, L.P., 2006. Phenotype of frailty: characterization in the Women's Health and Aging Studies. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 61, 262–266.

Barzilay, J.I., Blaum, C., Moore, T., Xue, Q.L., Hirsch, C.H., Walston, J.D., Fried, L.P., 2007. Insulin resistance and inflammation as precursors of frailty: the Cardiovascular Health Study. Archives of Internal Medicine 167, 635–641.

Bergman, H., Ferrucci, L., Guralnik, J., Hogan, D.B., Hummel, S., Karunananthan, S., Wolfson, C., 2007. Frailty: an emerging research and clinical paradigm - issues and controversies. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 62, 731–737.

Bischoff, C., Petersen, H.C., Graakjaer, J., Andersen-Ranberg, K., Vaupel, J.W., Bohr, V.A., Kolvraa, S., Christensen, K., 2006. No association between telomere length and survival among the elderly and oldest old. Epidemiology 17, 190–194.

Blaum, C.S., Xue, Q.L., Tian, J., Semba, R.D., Fried, L.P., Walston, J., 2009. Is hyperglycemia associated with frailty status in older women? Journal of the American Geriatrics Society 57, 840–847.

Ble, A., Cherubini, A., Volpato, S., Bartali, B., Walston, J.D., Windham, B.G., Bandinelli, S., Lauretani, F., Guralnik, J.M., Ferrucci, L., 2006. Lower plasma vitamin E levels are associated with the frailty syndrome: the InCHIANTI study. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 61, 278–283.

Bortz, W.M., 2002. A conceptual framework of frailty: a review. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 57, M283–M288.

Cappola, A.R., Xue, Q.-L., Fried, L.P., 2009. Multiple hormonal deficiencies in anabolic hormones are found in frail older women: the Women's Health and Aging Studies. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 64, 243–248.

Cawthon, P.M., Marshall, L.M., Michael, Y., Dam, T.-T., Ensrud, K.E., Barrett-Connor, E., Orwoll, E.S., Osteoporotic Fractures in Men Research Group, 2007. Frailty in older men: prevalence, progression, and relationship with mortality. Journal of the American Geriatrics Society 55, 1216–1223.

Cawthon, R.M., Smith, K.R., O'Brien, E., Sivatchenko, A., Kerber, R.A., 2003. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet 361, 393–395.

Chang, S.S., Weiss, C.O., Xue, Q.-L., Fried, L.P., 2010. Patterns of comorbid inflammatory diseases in frail older women: the Women's Health and Aging Studies I and II. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 65A, 407–413.

Chang, S.S., Weiss, C.O., Xue, Q.-L., Fried, L.P., 2012. Association between inflammatory-related disease burden and frailty: Results from the Women's Health and Aging Studies (WHAS) I and II. Archives of Gerontology and Geriatrics 54, 9–15.

Cigolle, C.T., Ofstedal, M.B., Tian, Z., Blaum, C.S., 2009. Comparing models of frailty: the Health and Retirement Study. Journal of the American Geriatrics Society 57, 830–839.

Collerton, J., Barrass, K., Bond, J., Eccles, M., Jagger, C., James, O., Martin-Ruiz, C., Robinson, L., von Zglinicki, T., Kirkwood, T., 2007. The Newcastle 85+ Study: biological, clinical and psychosocial factors associated with healthy ageing: study protocol. BMC Geriatrics 7, 14.

Collerton, J., Davies, K., Jagger, C., Kingston, A., Bond, J., Eccles, M.P., Robinson, L.A., Martin-Ruiz, C., von Zglinicki, T., James, O.F.W., Kirkwood, T.B.L., 2009. Health and disease in 85 year olds: baseline findings from the Newcastle 85+ cohort study. BMJ 339, b4904.

Davies, K., Collerton, J.C., Jagger, C., Bond, J., Barker, S.A.H., Edwards, J., Hughes, J., Hunt, J.M., Robinson, L., 2010. Engaging the oldest old in research: lessons from the Newcastle 85+ Study. BMC Geriatrics 10, 64.

De Fanis, U., Wang, G.C., Fedarko, N.S., Walston, J.D., Casolaro, V., Leng, S.X., 2008. T-Lymphocytes Expressing CC Chemokine Receptor-5 Are Increased in Frail Older Adults. Journal of the American Geriatrics Society 56, 904–908.

De Martinis, M., Franceschi, C., Monti, D., Ginaldi, L., 2006. Inflammation markers predicting frailty and mortality in the elderly. Experimental and Molecular Pathology 80, 219–227.

Derhovanessian, E., Larbi, A., Pawelec, G., 2009. Biomarkers of human immunosenescence: impact of Cytomegalovirus infection. Current Opinion in Immunology 21, 440–445.

Desquilbet, L., Jacobson, L.P., Fried, L.P., Phair, J.P., Jamieson, B.D., Holloway, M., Margolick, J.B., Multicenter Aids Cohort Study, 2007. HIV-1 infection is associated with an earlier occurrence of a phenotype related to frailty. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 62, 1279–1286.

Ensrud, K.E., Ewing, S.K., Taylor, B.C., Fink, H.A., Stone, K.L., Cauley, J.A., Tracy, J.K., Hochberg, M.C., Rodondi, N., Cawthon, P.M., for the Study of Osteoporotic Fractures Research Group, 2007. Frailty and risk of falls, fracture, and mortality in older women: the Study of Osteoporotic Fractures. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 62, 744–751.

Ferguson, F.G., Wikby, A., Maxson, P., Olsson, J., Johansson, B., 1995. Immune parameters in a longitudinal study of a very old population of Swedish people: a comparison between survivors and nonsurvivors. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 50, B378–B382.

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., 2001. Frailty in older adults: evidence for a phenotype. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 56, M146–M156.

Fried, L.P., Xue, Q.L., Cappola, A.R., Ferrucci, L., Chaves, P., Varadhan, R., Guralnik, J.M., Leng, S.X., Semba, R.D., Walston, J.D., Blaum, C.S., Bandeen-Roche, K., 2009. Nonlinear multisystem physiological dysregulation associated with frailty in older women: implications for etiology and treatment. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 64, 1049–1057.

Fulop, T., Larbi, A., Witkowski, J.M., McElhaney, J., Loeb, M., Mitnitski, A., Pawelec, G., 2010. Aging, frailty and age-related diseases. Biogerontology 11, 547–563.

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, 18–25.

Gruenewald, T.L., Seeman, T.E., Karlamangla, A.S., Sarkisian, C.A., 2009. Allostatic load and frailty in older adults. Journal of the American Geriatrics Society 57, 1525–1531.

Hogan, D.B., MacKnight, C., Bergman, H., on behalf of the Steering Committee Canadian Initiative on Frailty Aging, 2003. Models, definitions, and criteria of frailty. Aging Clinical and Experimental Research 15, 1–29.

Houben, J.M.J., Giltay, E.J., Rius-Ottenheim, N., Hageman, G.J., Kromhout, D., 2011. Telomere length and mortality in elderly men: the Zutphen Elderly Study. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 66, 38–44.

Hubbard, R.E., O'Mahony, M.S., Calver, B.L., Woodhouse, K.W., 2008. Nutrition, inflammation, and leptin levels in aging and frailty. Journal of the American Geriatrics Society 56, 279–284.

Hubbard, R.E., O'Mahony, M.S., Savva, G.M., Calver, B.L., Woodhouse, K.W., 2009. Inflammation and frailty measures in older people. Journal of Cellular and Molecular Medicine 13, 3103–3109.

Hummel, M., Abecassis, M.M., 2002. A model for reactivation of CMV from latency. Journal of Clinical Virology 25 (Suppl 2), S123–S136.

Kirkwood, T.B.L., 2008. A systematic look at an old problem. Nature 451, 644–647.

Kirkwood, T.B.L., 2011. Systems biology of ageing and longevity. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 366, 64–70.

Kregel, K.C., Zhang, H.J., 2007. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 292, R18–R36.

Kulminski, A.M., Ukraintseva, S.V., Akushevich, I.V., Arbeev, K.G., Yashin, A.I., 2007. Cumulative index of health deficiencies as a characteristic of long life. Journal of the American Geriatrics Society 55, 935–940.

Larbi, A., Franceschi, C., Mazzatti, D., Solana, R., Wikby, A., Pawelec, G., 2008. Aging of the immune system as a prognostic factor for human longevity. Physiology 23, 64–74.

Larbi, A., Kempf, J., Pawelec, G., 2007. Oxidative stress modulation and T cell activation. Experimental Gerontology 42, 852–858.

Le Couteur, D.G., Blyth, F.M., Creasey, H.M., Handelsman, D.J., Naganathan, V., Sambrook, P.N., Seibel, M.J., Waite, L.M., Cumming, R.G., 2010. The Association of Alanine Transaminase With Aging Frailty, and Mortality. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 65A, 712–717.

Leng, S., Chaves, P., Koenig, K., Walston, J., 2002. Serum interleukin-6 and hemoglobin as physiological correlates in the geriatric syndrome of frailty: a pilot study. Journal of the American Geriatrics Society 50, 1268–1271.

Leng, S.X., 2011. Role of chronic cytomegalovirus infection in T-cell immunosenescence and frailty: more questions than answers. Journal of the American Geriatrics Society 59, 2363–2365.

Leng, S.X., Cappola, A.R., Andersen, R.E., Blackman, M.R., Koenig, K., Blair, M., Walston, J.D., 2004a. Serum levels of insulin-like growth factor-I (IGF-I) and dehydroepiandrosterone sulfate (DHEA-S), and their relationships with serum interleukin-6, in the geriatric syndrome of frailty. Aging Clinical and Experimental Research 16, 153–157.

Leng, S.X., Yang, H., Walston, J.D., 2004b. Decreased cell proliferation and altered cytokine production in frail older adults. Aging Clinical and Experimental Research 16, 249–252.
Leng, S.X., Xue, Q.-L., Tian, J., Walston, J.D., Fried, L.P., 2007. Inflammation and frailty in older women. Journal of the American Geriatrics Society 55, 864–871.
Leng, S.X., Hung, W., Cappola, A.R., Yu, Q., Xue, Q.-L., Fried, L.P., 2009a. White blood cell counts, insulin-like growth factor-1 levels, and frailty in community-dwelling older women. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 64, 499–502.
Leng, S.X., Xue, Q.-L., Tian, J., Huang, Y., Yeh, S.-H., Fried, L.P., 2009b. Associations of neutrophil and monocyte counts with frailty in community-dwelling disabled older women: Results from the Women's Health and Aging Studies I. Experimental Gerontology 44, 511–516.
Leng, S.X., Qu, T., Semba, R.D., Li, H., Yao, X., Nilles, T., Yang, X., Manwani, B., Walston, J.D., Ferrucci, L., Fried, L.P., Margolick, J.B., Bream, J.H., 2011a. Relationship between cytomegalovirus (CMV) IgG serology, detectable CMV DNA in peripheral monocytes, and CMV pp65(495-503)-specific CD8+ T cells in older adults. AGE 33, 607–614.
Leng, S.X., Tian, X., Matteini, A., Li, H., Hughes, J., Jain, A., Walston, J.D., Fedarko, N.S., 2011b. IL-6-independent association of elevated serum neopterin levels with prevalent frailty in community-dwelling older adults. Age and Ageing 40, 475–481.
Lipsitz, L.A., 2002. Dynamics of stability: the physiologic basis of functional health and frailty. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 57, B115–B125.
Martin-Ruiz, C., Jagger, C., Kingston, A., Collerton, J., Catt, M., Davies, K., Dunn, M., Hilkens, C., Keavney, B., Pearce, S.H.S., den Elzen, W.P.J., Talbot, D., Wiley, L., Bond, J., Mathers, J.C., Eccles, M.P., Robinson, L., James, O., Kirkwood, T.B.L., von Zglinicki, T., 2011. Assessment of a large panel of candidate biomarkers of ageing in the Newcastle 85+ Study. Mechanisms of Ageing and Development 132, 496–502.
Martin-Ruiz, C.M., Gussekloo, J., van Heemst, D., von Zglinicki, T., Westendorp, R.G.J., 2005. Telomere length in white blood cells is not associated with morbidity or mortality in the oldest old: a population-based study. Aging Cell 4, 287–290.
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, 2201–2208.
Mitnitski, A., Song, X., Skoog, I., Broe, G.A., Cox, J.L., Grunfeld, E., Rockwood, K., 2005. Relative fitness and frailty of elderly men and women in developed countries and their relationship with mortality. Journal of the American Geriatrics Society 53, 2184–2189.
Mogensen, T.H., Paludan, S.R., 2001. Molecular pathways in virus-induced cytokine production. Microbiology and Molecular Biology Reviews 65, 131–150.
Molloy, D.W., Standish, T.I., 1997. A guide to the standardized Mini-Mental State Examination. International Psychogeriatrics 9 (Suppl 1), 87–94.
Office for National Statistics, 2004. 2001 Census. Standard tables for health areas.
Pawelec, G., Derhovanessian, E., Larbi, A., Strindhall, J., Wikby, A., 2009. Cytomegalovirus and human immunosenescence. Reviews in Medical Virology 19, 47–56.
Prlic, M., Sacks, J.A., Bevan, M.J., 2012. Dissociating markers of senescence and protective ability in memory T cells. PLoS ONE 7, e32576.
Prosch, S., Docke, W.D., Reinke, P., Volk, H.D., Kruger, D.H., 1999. Human cytomegalovirus reactivation in bone-marrow-derived granulocyte/monocyte progenitor cells and mature monocytes. Intervirology 42, 308–313.
Puts, M.T.E., Visser, M., Twisk, J.W.R., Deeg, D.J.H., Lips, P., 2005. Endocrine and inflammatory markers as predictors of frailty. Clinical Endocrinology 63, 403–411.
Reiner, A.P., Aragaki, A.K., Gray, S.L., Wactawski-Wende, J., Cauley, J.A., Cochrane, B.B., Kooperberg, C.L., Woods, N.F., LaCroix, A.Z., 2009. Inflammation and thrombosis biomarkers and incident frailty in postmenopausal women. The American Journal of Medicine 122, 947–954.
Rockwood, K., Andrew, M., Mitnitski, A., 2007. A comparison of two approaches to measuring frailty in elderly people. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 62, 738–743.
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, 722–727.
Rockwood, K., Mitnitski, A., Song, X., Steen, B., Skoog, I., 2006. Long-term risks of death and institutionalization of elderly people in relation to deficit accumulation at age 70. Journal of the American Geriatrics Society 54, 975–979.
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, E487–E494.
Rodríguez-Mañas, L., Féart, C., Mann, G., Viña, J., Chatterji, S., Chodzko-Zajko, W., Gonzalez-Colaço Harmand, M., Bergman, H., Carcaillon, L., Nicholson, C., Scuteri, A., Sinclair, A., Pelaez, M., Van der Cammen, T., Beland, F., Bickenbach, J., Delamarche, P., Ferrucci, L., Fried, L.P., Gutiérrez-Robledo, L.M., Rockwood, K., Rodríguez Artalejo, F., Serviddio, G., Vega, E., on behalf of the FODCC group, 2012. Searching for an operational definition of Frailty: a Delphi method based consensus statement. The Frailty operative definition-consensus conference project. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences (April 16 [epub ahead of print]).
Romero-Ortuno, R., O'Shea, D., Kenny, R.A., 2011. The SHARE frailty instrument for primary care predicts incident disability in a European population-based sample. Quality in Primary Care 19, 301–309.
Sanders, J.L., Boudreau, R.M., Fried, L.P., Walston, J.D., Harris, T.B., Newman, A.B., 2011. Measurement of organ structure and function enhances understanding of the physiological basis of frailty: the Cardiovascular Health Study. Journal of the American Geriatrics Society 59, 1581–1588.
Santos-Eggimann, B., Cuenoud, P., Spagnoli, J., Junod, J., 2009. Prevalence of frailty in middle-aged and older community-dwelling Europeans living in 10 countries. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 64, 675–681.
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, 747–754.
Searle, S.D., Mitnitski, A., Gahbauer, E.A., Gill, T.M., Rockwood, K., 2008. A standard procedure for creating a frailty index. BMC Geriatrics 8, 24.
Semba, R.D., Margolick, J.B., Leng, S., Walston, J., Ricks, M.O., Fried, L.P., 2005. T cell subsets and mortality in older community-dwelling women. Experimental Gerontology 40, 81–87.
Serviddio, G., Romano, A.D., Greco, A., Rollo, T., Bellanti, F., Altomare, E., Vendemiale, G., 2009. Frailty syndrome is associated with altered circulating redox balance and increased markers of oxidative stress. International Journal of Immunopathology and Pharmacology 22, 819–827.
Shardell, M., Hicks, G.E., Miller, R.R., Kritchevsky, S., Andersen, D., Bandinelli, S., Cherubini, A., Ferrucci, L., 2009. Association of low vitamin D levels with the frailty syndrome in men and women. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 64, 69–75.
Sternberg, S.A., Schwartz, A.W., Karunananthan, S., Bergman, H., Mark Clarfield, A., 2011. The identification of Frailty: a systematic literature review. Journal of the American Geriatrics Society 59, 2129–2138.
Szanton, S.L., Allen, J.K., Seplaki, C.L., Bandeen-Roche, K., Fried, L.P., 2009. Allostatic load and frailty in the Women's Health and Aging Studies. Biological Research for Nursing 10, 248–256.
Travison, T.G., Nguyen, A.-H., Naganathan, V., Stanaway, F.F., Blyth, F.M., Cumming, R.G., Le Couteur, D.G., Sambrook, P.N., Handelsman, D.J., 2011. Changes in Reproductive Hormone Concentrations Predict the Prevalence and Progression of the Frailty Syndrome in Older Men: The Concord Health and Ageing in Men Project. The Journal of Clinical Endocrinology and Metabolism 96, 2464–2474.
United Nations Department of Economic and Social Affairs Population Division, 2002. World Population Ageing: 1950–2050. United Nations, New York. Available from http://www.un.org/esa/population/publications/worldageing19502050/ (accessed 27/03/12).
von Zglinicki, T., Martin-Ruiz, C.M., 2005. Telomeres as biomarkers for ageing and age-related diseases. Current Molecular Medicine 5, 197–203.
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, 991–1001.
Walston, J., McBurnie, M.A., Newman, A., Tracy, R.P., Kop, W.J., Hirsch, C.H., Gottdiener, J., Fried, L.P., 2002. Cardiovascular Health Study Frailty and activation of the inflammation and coagulation systems with and without clinical comorbidities: results from the Cardiovascular Health Study. Archives of Internal Medicine 162, 2333–2341.
Wang, G.C., Kao, W.H.L., 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. American Journal of Epidemiology 171, 1144–1152.
Wikby, A., Ferguson, F., Forsey, R., Thompson, J., Strindhall, J., Lofgren, S., Nilsson, B.-O., Ernerudh, J., Pawelec, G., Johansson, B., 2005. An immune risk phenotype, cognitive impairment, and survival in very late life: impact of allostatic load in Swedish octogenarian and nonagenarian humans. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 60, 556–565.
Wikby, A., Mansson, I.A., Johansson, B., Strindhall, J., Nilsson, S.E., 2008. The immune risk profile is associated with age and gender: findings from three Swedish population studies of individuals 20–100 years of age. Biogerontology 9, 299–308.
Wikby, A., Maxson, P., Olsson, J., Johansson, B., Ferguson, F.G., 1998. Changes in CD8 and CD4 lymphocyte subsets T cell proliferation responses and non-survival in the very old: the Swedish longitudinal OCTO-immune study. Mechanisms of Ageing and Development 102, 187–198.
Wilhelm-Leen, E.R., Hall, Y.N., Deboer, I.H., Chertow, G.M., 2010. Vitamin D deficiency and frailty in older Americans. Journal of Internal Medicine 268, 171–180.
Woo, J., Tang, N.L.S., Suen, E., Leung, J.C.S., Leung, P.C., 2008. Telomeres and frailty. Mechanisms of Ageing and Development 129, 642–648.
Wu, I.C., Shiesh, S.-C., Kuo, P.-H., Lin, X.-Z., 2009. High oxidative stress is correlated with frailty in elderly chinese. Journal of the American Geriatrics Society 57, 1666–1671.