Immune-endocrine biomarkers as predictors of frailty and mortality

a 10-year longitudinal study in community-dwelling older people

Immune-endocrine biomarker as predictors of frailty and mortality: a ten year longitudinal study in community dwelling older people

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Abstract

Background

Frailty is a multidimensional geriatric syndrome characterised by a state of increased vulnerability to disease. Its causes are unclear, limiting opportunities for intervention. Age related changes to the immune-endocrine axis are implicated. This study investigated the associations between the immune-endocrine axis and frailty as well as mortality ten years later among men and women aged 65 to 70 years.

Methods

We studied 254 participants of the Hertfordshire Ageing Study at baseline and 10 year follow up. At baseline (1994/5) they completed a health questionnaire and had collection of blood samples for immune-endocrine analysis. At follow up (2003/5), frailty was characterised and mortality ascertained.

Findings

Higher baseline levels of white cell count (WCC), neutrophils, monocytes, lymphocytes, ESR, T4 and lower levels of DHEAS and higher cortisol:DHEAS ratio were all significantly associated with increased odds of frailty at follow-up. Higher baseline levels of ESR, neutrophils, monocytes and IL-1β were all associated with increased likelihood of all-cause mortality during the follow-up period. Baseline WCC and cortisol:DHEAS clearly discriminated between individuals who went on to be frail at follow-up.

Interpretation

We present the first evidence that immune-endocrine biomarkers are associated with the likelihood of frailty as well as mortality over a 10 year period. This augments our understanding of the aetiology of frailty and a suggests a screening programme at ages 60-70 years could help to identify individuals who are at high risk of becoming frail and who would benefit from early, targeted intervention, for example with DHEA supplementation or anti-inflammatory strategies. Progress towards the prevention of frailty would bring major health and socioeconomic benefits at the individual and the population level.

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Introduction

Frailty is a multidimensional geriatric syndrome (1); it may be described as a state of increased vulnerability which results from decreased physiological reserves, multi-system dysregulation and limited capacity to maintain homeostasis (2). Frailty is of increasing global importance, impacting on all forms of adult healthcare as well as socioeconomic policy. A person who is frail is less likely to be living independently at home and people who are frail have an increased likelihood of morbidity and mortality (3;4). Frailty also has predictive validity for adverse health outcomes including falls, disability and receipt of hospital care (4;5). A major healthcare challenge of the 21st century is to identify people at risk of becoming frail and intervene early.

The Fried criteria (4), the most widely implemented objective approach to the classification of frailty, define frailty on the basis of weight loss, weakness, exhaustion, slowness and low activity. Prevalence estimates were recently reported as 8·5% for women and 4·1% for men among community dwelling people aged 64 to 74 years (6). The numbers of frail older people will increase as populations age, particularly as numbers at the oldest ages are increasing the fastest.

Age-related changes in the immune-endocrine axis are implicated in a wide range of disease processes in older people, causing significant morbidity and mortality (7) . For example, white blood cells, are positively associated with cardiovascular disease, cancer and all-cause mortality (8). Levels of dehydroepiandosterone-sulphate (DHEAS) decline with age and these too are associated with increased morbidity including cardiovascular disease (9), osteoporosis (10) and all cause mortality (11). Relationships between low thyroid function

and lifespan in older people have also been noted and raised serum free T4 is associated with increased mortality in the oldest old (12).

These changes are also implicated in the frailty syndrome. Cross-sectional associations have been demonstrated with C-reactive protein (CRP), interleukin (IL)-6, total white-blood cell counts, neutrophils, monocytes, insulin-like growth factor-1 and DHEAS (13-17). However causality cannot be determined in cross-sectional studies and to date, only a small number of studies have examined longitudinal associations between inflammation and frailty (18;19). Few other biomarkers associated with the onset of frailty have yet been identified.

The objective of the current study was to investigate the associations between biomarkers of the immune-endocrine axis and frailty as well as all-cause mortality ten years later among community dwelling men and women aged 65 to 70 years.

Methods

The Hertfordshire Ageing Study (HAS) has been described previously (20). In brief, 717 men and women who were born in Hertfordshire, UK between 1920 and 1930 attended a home interview and clinic in 1994/5 where a wide range of markers of ageing were characterised. In 2003/5, a ten year follow-up was conducted; 359 men and women participated in a home interview of whom 254 attended a clinic at which Fried frailty was assessed (20). All-cause mortality was ascertained between the two phases.

At the 1994/5 baseline HAS home interview a trained research nurse ascertained smoking habit, alcohol intake, self-reported walking speed, medications, and current or most recent full-time occupation and husband's occupation for ever-married women. At the 1994/5 baseline HAS clinic, height and weight were measured. Grip strength was measured using a Jamar hand grip dynamometer. Venous blood was collected for laboratory automated haemoglobin, white cell counts, erythrocyte sedimentation rate (ESR), albumin, thyroid stimulating hormone (TSH), free T4 (T4), testosterone and sex-hormone binding globulin (SHBG) and serum stored at -80°C.

Serological Analysis

The serum free cytokines IL-1β, IL-6, IL-10 and TNF-α were simultaneously measured using commercially available multiplex luminometry whilst C-reactive protein was measured using singleplex luminometry (Invitrogen, UK). Detection of serum cortisol and DHEAS was completed using commercially available enzyme-linked immunosorbent assay kits, (IBL International, Germany) (REF).

Frailty was assessed at the 2003/5 follow-up. The Fried criteria define frailty as presence of three or more of the following: unintentional weight loss (greater than 10lb over the past year), weakness, self-reported exhaustion, slow walking speed and low physical activity (3). These were operationalised as previously in the Hertfordshire Cohort Study (4) and as follows: weakness was defined as a maximum grip strength of ≤30kg for men and ≤20kg for women (21); exhaustion was identified if the participant felt that everything they did was an effort for either moderate amounts or most of the time in the past week; slow walking speed was defined as a 3m walk time in the slowest fifth of the HAS sex-specific distribution (≥4·04 seconds for men and ≥4·54 seconds for women); and low physical activity was identified if the participant had a short-form 36 (SF-36) physical functioning score in the bottom fifth of the HAS sex-specific distribution (<45 for men and <33 for women).

Intra- and inter-observer studies were carried out during the fieldwork. The HAS had ethical approval from the Hertfordshire and Bedfordshire Local Research Ethics Committee and all participants gave written informed consent.

Statistical methods

Positively skewed biomarkers were $\log_e$ transformed to normal distributions as necessary. Weight and height were positively correlated (men r=0·53,p<0·001; women r=0·31,p<0·001); to avoid multicolinearity problems, a standardised residual of weight adjusted for height was derived. Follow-up time was calculated as time elapsed in decimal years between the HAS 1994/5 baseline and 2003/5 follow-up clinics. Pearson's correlation coefficients and principal components analysis were used to explore the inter-relationships between the immune-endocrine biomarkers. Cox's proportional hazards models and logistic regression models were used to analyse the associations between the 1994/5 baseline panel of biomarkers and

all-cause mortality between data collection points and Fried frailty at the 2003/5 follow-up respectively. Analyses were conducted with and without adjustment for the potential confounding effects of gender, follow-up duration (for logistic models for frailty only), and 1994/5 baseline age, height, weight for height, smoking status, alcohol consumption, social class in adulthood, walking speed, and number of systems medicated. A 5% significance level was principally used to identify statistically significant associations but a Bonferroni correction was also applied to enable identification of significant associations after allowance for multiple comparisons. Receiver-operating characteristic (ROC) curves were used to explore the ability of the 1994/5 baseline biomarkers to discriminate between frailty status at follow-up in 2003/5. Data were principally analysed for men and women combined but analyses were repeated for men and women separately. All analyses were conducted using Stata 11 (Stata Statistical Software, StataCorp, 2009).

Results

411 men and 306 women participated in the 1994/5 baseline clinic; 81 men (19·7%) and 40 women (13·1%) had died by the time of the 2003/5 follow-up (hazard ratio [HR] for men compared with women 1·51, 95% confidence interval [95%CI] 1·03, 2·21, p=0·03; total person years of follow-up 5,971).

Table 1 shows the baseline summary characteristics of the 153 men and 101 women who participated in the 1994/5 baseline clinic and who were also assessed for Fried frailty status at 2003/5 follow-up. The average age at the 1994/5 baseline clinic was 66·9 years for men and 67·3 years for women. The median follow-up time was 10·4 years for men and 9·9 years for women. At the 2003/5 follow-up the prevalence of Fried frailty was 5·2% for men and 11·9% for women (p=0·05 for gender difference). Immune biomarkers were strongly inter-correlated: the pairwise correlation coefficients between white cell count and each of

neutrophils, monocytes and lymphocytes ranged from 0·58 to 0·91, all p<0·0001. In addition, neutrophils and monocytes were strongly correlated with one another (r=0·58, p<0·0001) and CRP was correlated with each of white cell count, neutrophils, monocytes and ESR (correlation coefficients ranged from 0·33 to 0·37, all p<0·0001). However, the endocrine markers cortisol and DHEAs were independent of one another (r=0·01, p=0·90) and of all the other biomarkers considered in Table 1 (correlations ranged from 0·02 to 0·15, p≥0·03 for all) with the exception of the ratio of cortisol to DHEAs of which they are a component. A principal components analysis for the biomarkers in table 1 confirmed these patterns of association: the first principal component accounted for 19% of the variation in the data and was a weighted average of white cell count, neutrophils, monocytes, lymphocytes and CRP; the second component accounted for 12% of the variation in the data and was a contrast between cortisol and DHEAs.

Table 2 presents the associations between the panel of biomarkers as measured for 717 men and women at the 1994/5 clinic and all-cause mortality between HAS follow-ups for men and women combined. In unadjusted analyses, an SD increase in levels of ESR (HR [95%CI] 1·33 [1·11,1·58], p=0·002), neutrophils (HR[95% CI] 1·33 [1·11,1·59], p=0·002), monocytes (HR[95% CI] 1·19 [1·00,1·43], p=0·05) and IL-1β (HR[95% CI] 1·17 [1·00,1·36], p=0·04) were all associated with increased likelihood of mortality between data collection points. The associations between ESR and neutrophils and mortality were also significant after Bonferroni correction (p<0·003). The results were unaltered by adjustment for gender and age at 1994/5 baseline (table 2). However, the associations between monocytes, IL-1β and mortality were attenuated by also adjusting for height, weight for height, smoking, alcohol, social class, walking speed, and number of systems medicated, all as ascertained in 1994/5 baseline.

Table 3 presents the associations between the panel of biomarkers as measured at the 1994/5 baseline and Fried frailty status at 2003/5 follow-up for men and women combined. In unadjusted analyses, an SD increase in levels of white cell count (odds ratio OR[95%CI] 2·51 [1·49,4·24], p=0·001), ESR (OR[95%CI] 2·23 [1·28,3·88], p=0·005), neutrophils (OR[95% CI] 2·17 [1·29,3·65], p=0·004), monocytes (OR[95% CI] 3·22 [1·80,5·74], p<0·001), lymphocytes (OR[95% CI] 2·00 [1·23,3·25], p=0·005), T4 (OR[95% CI] 1·86 [1·11,3·13], p=0·02) and ratio of cortisol to DHEAS (OR[95%CI] 1·78 [1·17,2·72], p=0·007), and an SD decrease in levels of DHEAS (OR[95% CI] 2·04 [1·30,3·23] p=0·002),were all associated with increased odds of frailty at 2003/5 follow-up. The associations between white cell count, monocytes and DHEAS and frailty were also significant after Bonferroni correction (p<0·003). The results were unaltered by adjustment for gender, age and duration of follow-up (table 3). However, the associations between ESR (p=0·05) and T4 (p=0·07) were attenuated by also adjusting for height, weight for height, smoking, alcohol, social class and walking speed. The associations between white cell count, monocytes, lymphocytes, DHEAS, and ratio of cortisol to DHEAS with frailty were little altered by additionally adjusting for number of systems medicated at the 1994/5 baseline as a marker of co-morbidity. The prevalence of frailty according to thirds of the distribution of white cell count, neutrophils, monocytes, lymphocytes, DHEAS, and ratio of cortisol to DHEAS is shown in Figure 1.

Receiver operating characteristic (ROC) curves were used to explore the ability of white cell count (an immune marker) and the ratio of cortisol to DHEAS (an endocrine marker) at the 1994/5 baseline to discriminate between frail and non-frail individuals at the 2003/5 follow-up. The ROC areas under the curve were 0·70 (95%CI 0·55, 0·84) for white cell count and 0·72 (95%CI 0·59, 0·86) for ratio of cortisol to DHEAS; both areas under the curve are suggestive of fair-good discrimination between frail and non-frail individuals. The optimal categorisations for discrimination between frail and non-frail individuals were $\geq 6x10^9$/L versus $<6x10^9$/L for white cell count (frailty prevalence 12·1% versus 4·6% respectively), and

≥0·172 versus <0·172 for ratio of cortisol to DHEAS (frailty prevalence 12·6% versus 3·8%). Further discrimination was achieved by combining the categorisations of white cell count and ratio of cortisol to DHEAS; the prevalence of frailty at 2003/5 follow-up among individuals who had a white cell count $<6x10^9$/L and a ratio of cortisol to DHEAS <0·172 at 1994/5 baseline was only 2·0% in contrast with a prevalence of 8·0% among individuals who had only one of white cell count $\geq 6x10^9$/L or ratio ≥0·172, and a frailty prevalence of 17·1% among individuals with white cell count $\geq 6x10^9$/L and ratio of cortisol to DHEAS ≥0·172.

Results were similar for men and women analysed separately (data not shown), but the strength of statistical significance was lower for all associations owing to limited power from smaller sample sizes.

Discussion

We have shown that higher baseline levels of total WCC, neutrophils, monocytes, lymphocytes, ESR and free T4, and lower levels of DHEAS as well as higher ratios of cortisol to DHEAS were all associated with an increased likelihood of being frail at ten year follow-up in a cohort of community dwelling older people. Higher baseline levels of ESR, neutrophils, monocytes and IL-1β were associated with increased likelihood of all-cause mortality across the ten years.

We have also demonstrated that a single baseline marker of the immune axis (WCC), combined with a baseline marker of the endocrine axis (cortisol:dheas), clearly discriminated between individuals who went on to be frail at ten year follow-up . If validated in other populations, our results suggest that a screening programme at ages 60-70 years could help

to identify individuals who are at high risk of becoming frail and who would benefit from early, targeted intervention. This might include comprehensive geriatric assessment, medical, nutritional and exercise treatments (22;23). Pharmacological interventions are also being trialled (13;24). Furthermore, these biomarkers are simple, inexpensive and routinely used in clinical practice; they may be combined with other markers (e.g. grip strength, sarcopenia) to increase accuracy and generate a functional predictive tool of clinical value. This has implications in-terms of healthcare planning and policy making and high potential of significant social, economic and well-being benefits at both individual and global population levels.

These findings complement and add to cross-sectional studies by Leng and colleagues who showed that high neutrophil and monocytes counts were associated with frailty in disabled older women (15) and also Voznesensky and colleagues who demonstrated associations with DHEAS in community dwelling older people (14). This is the first study to show longitudinal associations with frailty across both the immune and endocrine axis, suggesting that the age associated changes to the cellular immune system and hypothalamic-pituitary-adrenal (HPA) axis are central to the rate of development of age-associated diseases and, as a consequence, to frailty and mortality.

White blood cells are important co-ordinators of inflammation; the age associated decline of the immune system, immunesenescence, is well documented (25). These changes contribute to inflamm-aging, a progressive increase in pro-inflammatory status with age (26), and potentially an important mechanism explaining the association of white cells with prevalent frailty and mortality. Associations between specific pro-inflammatory cytokines or CRP and frailty have not been shown in this study; this may reflect the complexities of

inflammation which involves an inflammatory milieu of multiple proteins rather than a specific inflammatory protein.

Both cortisol and the DHEAS are outputs of the HPA axis; cortisol has mainly immunosuppressive actions while DHEAS is immune enhancing (25). With ageing there is increasing imbalance between the two hormones, due to reducing DHEAS levels from age 30 years onwards (adrenopause), and relatively stable cortisol levels (27). Raised levels of thyroid hormone are thought to further modulate the HPA axis through central changes in sensitivity to corticotrophin releasing hormone (28). These changes are believed to contribute to the process of immunesenescence (29). Furthermore, cortisol and DHEAS have direct effects on frailty via interaction with anabolic and catabolic pathways within myocytes. Importantly, this ratio can be manipulated via pharmacological supplementation with DHEA to raise serum DHEAS levels. A recent randomised controlled trail by Kenny and colleagues demonstrated that DHEA supplementation improved sarcopenia in already frail women involved in gentle exercise when compared to placebo (24).

Our study has some limitations. Firstly, we cannot completely exclude the effects of co-existing sub-clinical infections at the time of immune-endocrine analysis. However, participants were presumed fit and able to attend clinic appointments for data collection and results were screened prior to analysis for patterns suggestive of acute infection or haematological malignancy and four results removed from the data set. Secondly, study participants were lost to follow-up between the 1994/5 baseline and 2003/5 follow-up clinics due to a variety of reasons (including mortality, loss to follow-up, refusal to participate) and we have previously shown that a healthy participant effect is, unsurprisingly, evident in HAS (20). In the current study, the 153 men who went on to have frailty classified at 2003/5 HAS follow-up were significantly ($p<0{\cdot}05$) younger, less likely to be current smokers, were of higher social class, and had lower white cell count, ESR, neutrophil, monocytes, T4, IL-6 and

CRP levels at the 1994/5 clinic than the 258 men who only participated in the 1994/5 baseline study. Selection effects were less evident for women; the 101 women who went on to have frailty classified at 2003/5 HAS follow-up were significantly ($p<0{\cdot}05$) less likely to be current smokers and had lower neutrophil levels at the 1994/5 clinic than the 205 women who only participated in the 1994/5 study. These selection effects have the potential to bias our results. However, our analyses were internal to the HAS sample; bias would only be introduced if the associations between biomarkers and frailty were systematically different among those who participated in our study, and those who did not; this seems unlikely. Finally, there are numerous candidate biomarkers within the immune-endocrine axis that we did not analyse and there is scope to widen this biomarker battery. However this may add unnecessary complexity and we specifically chose readily available biomarkers to facilitate easy translation into the clinical environment.

In conclusion, we present the first evidence that immune-endocrine biomarkers can predict the likelihood of frailty as well as mortality over a 10 year period. This augments our understanding of the aetiology of frailty and suggests the potential for screening and early identification and intervention in those at high risk. Progress towards the prevention of frailty would bring major health and socioeconomic benefits both at the individual and the population level. Further work is needed to replicate these findings in other studies and to elucidate the underlying mechanisms to develop the necessary evidence base for successful intervention strategies.

Funding

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Conflicts of interest

None declared.

Table 1. Baseline (1994/5) characteristics of participants in the 2003/5 HAS follow-up

Mean (SD)	Men (n=153)	Women (n=101)
Age (years)	66·9 (2·2)	67·3 (2·1)
Height (cm)	172·6 (6·3)	159·8 (5·1)
Weight (kg)	80·4 (12·2)	69·2 (10·6)
Current smoker[a]	19 (12·4)	9 (8·9)
Moderate or higher weekly alcohol intake[a,b]	40 (26·1)	9 (8·9)
Non-manual social class[a,c]	73 (48·3)	43 (43·4)
Fairly brisk or fast walking speed[a]	42 (27·5)	26 (25·7)
Number of systems medicated[d]	1 (0,2)	1 (0,2)
White cell count (x10^9/L) [d]	5·5 (4·8, 6·6)	5·5 (4·7, 6·5)
ESR (mm/hr)[d]	6 (4,10)	14 (8,20)
Neutrophils (x10^9/L) [d]	3·4 (2·8,4·0)	3·2 (2·5,4·0)
Monocytes (x10^9/L) [d]	0·4 (0·3,0·5)	0·3 (0·3,0·4)
Lymphocytes (x10^9/L) [d]	1·6 (1·3, 2·0)	1·7 (1·4,2·1)
Albumin (g/L)	42·7 (2·0)	42·3 (2·0)
SHBG (nmol/L) [d]	36·3 (27·9,48·3)	51·2 (34·2,79·2)
Testosterone (nmol/L) [d]	16·3 (11·1,20·3)	No data
Haemoglobin (g/L)	14·5 (1·0)	13·5 (0·9)
TSH (mu/L) [d]	1·7 (1·2,2·3)	2·1 (1·5,3·3)
T4 (pmol/L) [d]	14·2 (13·2,15·6)	13·7 (12·6,15·3)
IL-1B (pg/ml) [d]	11·3 (5·6,17·7)	18·3 (11·7,26·2)
IL-6 (pg/ml) [d]	1·2 (0·2,2·0)	0·9 (0·4,2·0)
IL-10 (pg/ml)[d]	2·3 (2·5,4·7)	1·9 (0·3,2·1)
CRP (mg/l) [d]	1·9 (0·9,4·0)	3·1 (1·1,5·6)
DHEAS (nmol/l) [d]	2181 (1734,2888)	1495 (797,2261)
Cortisol (nmol/l) [d]	308 (235,385)	274 (210,349)
Ratio of Cortisol to DHEAS[d]	0·12 (0·09, 0·17)	0·19 (0·10, 0·38)
Follow-up time (years)[d]	10·4 (10·2,10·5)	9·9 (9·8,10·1)

Fried frailty[a,e]	8 (5·2)	12 (11·9)

[a]Number and percentage [b] Defined as weekly alcohol consumption of ≥11 units for men and ≥8 units for women

[c] Defined as classes I, II and IIINM of the 1990 OPCS coding of social class. Social class was identified on the basis of own current/most recent full-time occupation for men and never-married women, and from husband's occupation for ever-married women [d] Variable was positively skewed; median and inter-quartile range presented [e]Assessed at the 2003/5 follow-up

Table 2. Associations between 1994/5 biomarkers and all-cause mortality between HAS follow-ups for men and women combined

Biomarker	N	HR[a]	95%CI	*p*	HR[b]	95%CI	*p*	HR[c]	95%CI	*p*	HR[d]	95%CI	*p*
White cell count ($x10^9$/L)	697	1·18	(0·98,1·42)	*0·081*	1·17	(0·98,1·41)	*0·089*	1·15	(0·96,1·39)	*0·133*	1·15	(0·95,1·38)	*0·155*
ESR (mm/hr)	708	1·33	(1·11,1·58)	*0·002*	1·31	(1·10,1·56)	*0·002*	1·22	(1·01,1·48)	*0·037*	1·22	(1·01,1·47)	*0·041*
Neutrophils ($x10^9$/L)	708	1·33	(1·11,1·59)	*0·002*	1·31	(1·09,1·57)	*0·003*	1·21	(1·00,1·47)	*0·046*	1·21	(1·00,1·46)	*0·054*
Monocytes ($x10^9$/L)	708	1·19	(1·00,1·43)	*0·054*	1·19	(0·99,1·42)	*0·064*	1·13	(0·93,1·36)	*0·214*	1·12	(0·93,1·35)	*0·235*
Lymphocytes ($x10^9$/L)	708	1·10	(0·91,1·32)	*0·319*	1·11	(0·93,1·33)	*0·261*	1·06	(0·88,1·28)	*0·560*	1·07	(0·88,1·29)	*0·490*
Albumin (g/L)	708	0·93	(0·77,1·11)	*0·401*	0·92	(0·77,1·11)	*0·386*	0·94	(0·78,1·12)	*0·480*	0·94	(0·79,1·12)	*0·488*
SHBG (nmol/L)	689	0·93	(0·77,1·11)	*0·411*	0·93	(0·78,1·11)	*0·433*	0·95	(0·79,1·14)	*0·587*	0·98	(0·82,1·18)	*0·856*
Testosterone (nmol/L)[e]	711	1·18	(0·98,1·42)	*0·075*	1·16	(0·97,1·40)	*0·108*	1·15	(0·94,1·40)	*0·172*	1·14	(0·94,1·39)	*0·193*
Haemoglobin (g/L)	408	0·98	(0·78,1·24)	*0·894*	0·98	(0·78,1·24)	*0·888*	0·99	(0·76,1·28)	*0·937*	1·00	(0·77,1·30)	*0·989*
TSH (mu/L)	708	0·94	(0·78,1·13)	*0·521*	0·96	(0·80,1·15)	*0·655*	0·96	(0·80,1·15)	*0·643*	0·97	(0·81,1·17)	*0·783*
T4 (pmol/L)	696	0·95	(0·79,1·14)	*0·609*	0·96	(0·80,1·15)	*0·664*	1·00	(0·83,1·20)	*0·979*	1·00	(0·83,1·20)	*0·987*
IL-1B (pg/ml)	705	1·17	(1·00,1·36)	*0·044*	1·18	(1·00,1·38)	*0·045*	1·16	(0·98,1·37)	*0·085*	1·14	(0·96,1·35)	*0·149*
IL-6 (pg/ml)	504	0·97	(0·78,1·20)	*0·760*	0·98	(0·79,1·22)	*0·857*	0·96	(0·77,1·19)	*0·703*	0·96	(0·77,1·19)	*0·706*
IL-10 (pg/ml)	439	1·09	(0·87,1·37)	*0·431*	1·09	(0·87,1·37)	*0·459*	1·02	(0·81,1·29)	*0·846*	1·02	(0·81,1·28)	*0·882*
CRP (pg/ml)	537	0·95	(0·78,1·17)	*0·655*	0·95	(0·78,1·17)	*0·647*	0·98	(0·80,1·20)	*0·834*	0·98	(0·80,1·21)	*0·875*
DHEAS (nmol/l)	673	1·18	(0·97,1·43)	*0·091*	1·19	(0·98,1·44)	*0·083*	1·12	(0·92,1·37)	*0·262*	1·10	(0·90,1·35)	*0·354*
Cortisol (nmol/l)	692	1·08	(0·90,1·31)	*0·409*	1·08	(0·89,1·31)	*0·426*	1·09	(0·90,1·32)	*0·395*	1·08	(0·89,1·30)	*0·430*
Ratio of cortisol:DHEAS	689	1·04	(0·86,1·26)	*0·668*	1·04	(0·86,1·25)	*0·710*	1·02	(0·84,1·24)	*0·814*	1·00	(0·82,1·21)	*0·978*

N, number; HR, hazard ratio; CI, confidence interval; *p*, p-value

[a]Unadjusted hazard ratio for mortality per SD (standard deviation) increase in biomarker. [b]Hazard ratio adjusted for gender and baseline age

[c]Hazard ratio adjusted as in [b] and also for baseline height, weight for height, smoking, alcohol, social class and waking speed

[d]Hazard ratio adjusted as in [c] and also adjusted for baseline number of systems medicated as a marker of comorbidity

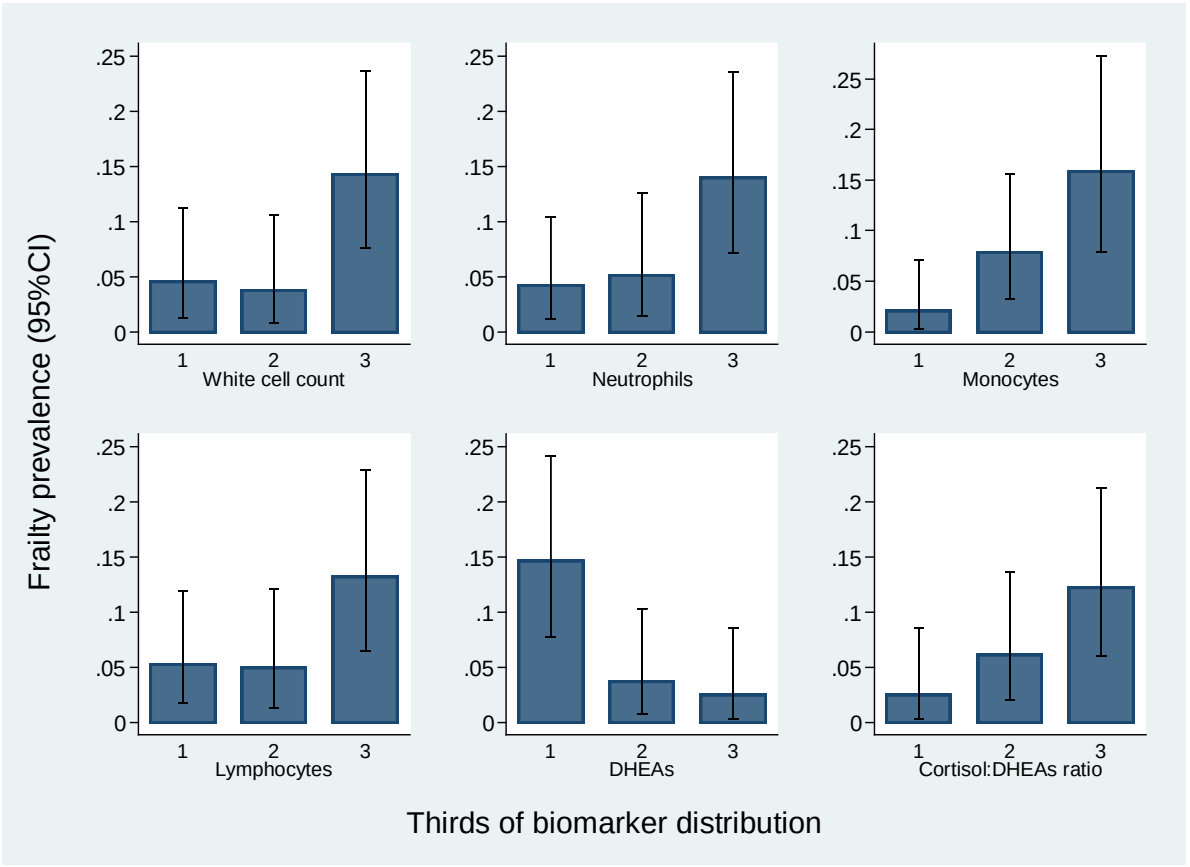
[e] Based on data for men only

Table 3. Associations between 1994/5 biomarkers and Fried frailty status at 2003/5 follow-up for men and women combined

Biomarker	N	OR[a]	95%CI	*p*	OR[b]	95%CI	*p*	OR[c]	95%CI	*p*	OR[d]	95%CI	*p*
White cell count (x10^9/L)	252	2·51	(1·49,4·24)	*0·001*	2·85	(1·60,5·07)	*<0·001*	2·36	(1·24,4·51)	*0·009*	2·22	(1·14,4·33)	*0·019*
ESR (mm/hr)	246	2·23	(1·28,3·88)	*0·005*	2·36	(1·29,4·30)	*0·005*	2·04	(0·99,4·21)	*0·054*	1·77	(0·85,3·65)	*0·125*
Neutrophils (x10^9/L)	252	2·17	(1·29,3·65)	*0·004*	2·42	(1·37,4·25)	*0·002*	1·92	(1·05,3·52)	*0·035*	1·83	(0·96,3·52)	*0·068*
Monocytes (x10^9/L)	252	3·22	(1·80,5·74)	*0·000*	4·11	(2·11,7·99)	*0·000*	4·23	(1·94,9·22)	*0·000*	3·88	(1·73,8·69)	*0·001*
Lymphocytes (x10^9/L)	252	2·00	(1·23,3·25)	*0·005*	2·07	(1·26,3·40)	*0·004*	2·00	(1·14,3·53)	*0·016*	2·09	(1·15,3·79)	*0·016*
Albumin (g/L)	251	0·70	(0·44,1·10)	*0·123*	0·66	(0·41,1·06)	*0·085*	0·59	(0·34,1·02)	*0·058*	0·48	(0·26,0·91)	*0·024*
SHBG (nmol/L)	252	0·98	(0·63,1·52)	*0·936*	0·90	(0·57,1·40)	*0·631*	0·89	(0·51,1·55)	*0·678*	0·88	(0·48,1·61)	*0·688*
Testosterone (nmol/L)[e]	151	1·54	(0·70,3·42)	*0·285*	1·06	(0·44,2·56)	*0·892*	4·06	(0·81,20·39)	*0·089*	5·42	(0·95,30·87)	*0·057*
Haemoglobin (g/L)	252	0·84	(0·52,1·37)	*0·495*	0·84	(0·50,1·42)	*0·522*	0·69	(0·39,1·22)	*0·203*	0·82	(0·46,1·48)	*0·515*
TSH (mu/L)	247	0·75	(0·48,1·17)	*0·202*	0·72	(0·45,1·16)	*0·178*	0·73	(0·42,1·26)	*0·254*	0·68	(0·39,1·20)	*0·186*
T4 (pmol/L)	250	1·86	(1·11,3·13)	*0·019*	2·08	(1·16,3·72)	*0·014*	1·78	(0·95,3·32)	*0·070*	1·41	(0·74,2·67)	*0·296*
IL-1B (pg/ml)	180	0·92	(0·51,1·63)	*0·764*	0·94	(0·51,1·72)	*0·839*	0·81	(0·43,1·54)	*0·525*	0·76	(0·40,1·44)	*0·400*
IL-6 (pg/ml)	151	1·75	(0·87,3·54)	*0·116*	1·93	(0·90,4·16)	*0·092*	1·65	(0·73,3·76)	*0·231*	1·64	(0·71,3·76)	*0·243*
IL-10 (pg/ml)	194	0·58	(0·29,1·14)	*0·116*	0·55	(0·26,1·18)	*0·124*	0·47	(0·17,1·29)	*0·142*	0·63	(0·22,1·83)	*0·400*
CRP (pg/ml)	243	1·49	(0·88,2·51)	*0·138*	1·59	(0·88,2·86)	*0·123*	1·18	(0·58,2·42)	*0·649*	1·04	(0·49,2·18)	*0·926*
DHEAS (nmol/l)	246	0·49	(0·31,0·77)	*0·002*	0·45	(0·27,0·74)	*0·002*	0·42	(0·23,0·74)	*0·003*	0·50	(0·27,0·91)	*0·023*
Cortisol (nmol/l)	246	1·04	(0·63,1·74)	*0·869*	1·08	(0·63,1·84)	*0·789*	1·07	(0·59,1·94)	*0·824*	1·14	(0·60,2·16)	*0·699*
Ratio of cortisol:DHEAS	246	1·78	(1·17,2·72)	*0·007*	2·03	(1·25,3·30)	*0·004*	2·02	(1·20,3·41)	*0·008*	1·79	(1·03,3·10)	*0·037*

N, number; OR, odds ratio; CI, confidence interval; *p*, p-value
[a]Unadjusted odds ratio for frailty per SD (standard deviation) increase in biomarker. [b]Odds ratio adjusted for gender, baseline age and follow-up duration
[c]Odds ratio adjusted as in [b] and also for baseline height, weight for height, smoking, alcohol, social class and waking speed
[d]Odds ratio adjusted as in [c] and also adjusted for baseline number of systems medicated as a marker of comorbidity
[e] Based on data for men only

Figure 1. Prevalence of Fried frailty at 2003/5 follow-up for men and women combined according to biomarker distribution at 1994/5 baseline



Covering letter:

Dear Dr Horton,

Re: Immune-endocrine biomarkers of frailty and mortality: a ten year longitudinal study in community dwelling older people

We would be grateful if you would consider the above research article for publication in your journal. We believe that it would be of considerable interest and relevance to your general and specialist international readership. Frailty is a well known term and commonly used in everyday language. It is also frequently encountered in the day-to-day practice of clinicians looking after older people. Its prevalence is increasing worldwide with population ageing and a decade has now passed since Linda Fried and colleagues first described the frailty phenotype. Despite this, studies of frailty are rarely reported in general medical journals.

In this paper we present the novel and exciting epidemiological findings that immune-endocrine biomarkers can predict the likelihood of frailty as well as mortality over a 10 year period. This is important because it augments our understanding of the aetiology of frailty and suggests the potential for a screening programme, early identification and intervention in individuals at high risk. Progress towards the prevention of frailty would bring major health and socioeconomic benefits both at the individual and the population level.

Thank you for considering our paper. We look forward to hearing from you.

Yours sincerely

D Baylis, D Bartlett, H Syddall, G Ntani, C Gale, C Cooper, J Lord, A Aihie Sayer

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