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RESEARCH PAPER

Associations between inflammation, cardiovascular biomarkers and incident frailty: the British Regional Heart Study

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Abstract

Introduction: cardiovascular disease (CVD) and chronic inflammation are implicated in the development of frailty. Longitudinal analyses of inflammatory markers, biomarkers of cardiac dysfunction and incidence of frailty are limited.
Methods: in the British Regional Heart Study, 1,225 robust or pre-frail men aged 71–92 years underwent a baseline examination, with questionnaire-based frailty assessment after 3 years. Frailty definitions were based on the Fried phenotype. Associations between incident frailty and biomarkers of cardiac dysfunction (high-sensitivity cardiac troponin T (hs-cTnT), N-terminal pro B-type natriuretic peptide (NT-proBNP)) and inflammation (C-reactive protein (CRP) and interleukin-6 (IL-6)) were examined, by tertile, with the lowest as reference.
Results: follow-up data were available for 981 men. Ninety one became frail. Adjusted for age, pre-frailty, prevalent and incident CVD, comorbidity, polypharmacy and socioeconomic status, IL-6 (third tertile OR 2.36, 95% CI 1.07–5.17) and hs-cTnT (third tertile OR 2.24, 95% CI 1.03–4.90) were associated with increased odds of frailty. CRP (third tertile OR 1.83, 95% CI 0.97–4.08) and NT-proBNP (second tertile OR 0.48, 95% CI 0.23–1.01) showed no significant association with incident frailty. The top tertiles of CRP, IL-6, hscTnT and NT-proBNP were strongly associated with mortality prior to follow-up.
Conclusion: IL-6 is associated with incident frailty, supporting the prevailing argument that inflammation is involved in the pathogenesis of frailty. Cardiomyocyte injury may be associated with frailty risk. Associations between elevated CRP and frailty cannot be fully discounted; NT-proBNP may have a non-linear relationship with incident frailty. CRP, IL-6, hs-cTnT and NT-proBNP are vulnerable to survivorship bias.

Keywords: inflammation, biomarkers, pro-brain natriuretic peptide (1–76), troponin T, frailty, aging, older people

Key Points

- Longitudinal studies of inflammation and frailty are limited, as are those with newer cardiovascular biomarkers.
- We tested associations between inflammation (C-reactive protein (CRP) and interleukin-6 (IL-6)), cardiovascular biomarkers (high-sensitivity cardiac troponin T (hs-cTnT), N-terminal pro B-type natriuretic peptide (NT-proBNP)) and incident frailty.
- IL-6 and hs-cTnT were associated with incident frailty; CRP and NT-proBNP were not.
- Inflammation, as measured by IL-6, may be linked to the development of frailty, as might myocardiocyte injury (hs-cTnT).
- All four biomarkers were strongly associated with mortality prior to follow-up, which may attenuate these associations.

Introduction

The frailty syndrome describes a constellation of features that predispose to ill health, loss of function and vulnerability to external insults [1]. Frailty is closely associated with comorbidities, including cardiovascular diseases (CVDs), which may share common pathophysiological determinants with frailty, including neuroendocrine disturbances and chronic inflammation [2].

There is evidence of a bidirectional relationship between CVD and frailty. In longitudinal studies, prevalent frailty is associated with greater risk of developing CVD, [3] and a more limited literature [4] suggests that CVD [5,6], and traditional cardiovascular risk scores [7,8], predict increased likelihood of incident frailty.

Whilst CVD and frailty appear intertwined, the underlying mechanisms connecting the two are less well characterised. Inflammation is strongly linked with frailty [9] and CVD [10] in cross-sectional analyses, and has been argued to be important in the processes of accelerated aging and frailty [11]. However, whilst elevated levels of the pro-inflammatory cytokine interleukin-6 (IL-6) predict worse physical function [12] and mobility limitation, [13] longitudinal studies of inflammation and incident frailty in older age have not found an association, with a meta-analysis of four papers [9,14–17] finding no statistically significant association between elevated C-reactive protein (CRP, an inflammatory biomarker) or IL-6 and incident frailty. A more recent analysis of a cohort of older Australian men also reported no relationship between IL-6 and incident frailty at 3 years, despite a strong cross-sectional association between frailty and IL-6 [18].

Amino-terminal pro B-type natriuretic peptide (NT-proBNP) and high-sensitivity cardiac troponin T (hs-cTnT) are in widespread clinical use as markers of cardiac stress and myocardial damage, respectively, but elevations are also seen in a range of non-cardiac illnesses [19,20] and associations have been reported, in cross-sectional analyses, with frailty [21–23]. Both biomarkers predict cardiovascular morbidity and overall mortality in older people, even in those who are apparently healthy. [24,25] Elevated NT-proBNP levels in midlife [12] and very old age [25] predict reduced physical functional ability, and elevated hs-cTnT levels predict future risk of hospitalisation for dementia [26]. However, to our knowledge, there have been no published longitudinal data on the relationship between these biomarkers and incident frailty. Understanding the association between cardiovascular biomarkers, inflammation and frailty could provide further understanding of common pathophysiological pathways.

We therefore aimed to determine the relationship between incident frailty, cardiovascular biomarkers (hs-cTnT and NT-proBNP) and inflammatory biomarkers (CRP and IL-6) in a cohort of older men.

Methods

All data were derived from the British Regional Heart Study, a prospective study of 7,735 men, aged 40–59 years at enrolment, drawn from one general practice in each of 24 British towns and socioeconomically representative of those areas. Over 99% of participants were of White European ethnicity [27]. Initial screening occurred in 1978–1980; 7,735 men (78% response rate) were recruited and examined at study entry. The cohort has been followed up for morbidity using primary care records, mortality using the NHS Central Register, and has had periodic postal questionnaires as well as in-person re-examinations. In 2010–2012, all 3,137 surviving men were invited to attend a 30-year re-examination. Respondents completed a questionnaire, underwent physical examination and provided a fasting blood sample [28]. Incident frailty was derived from a 3-year follow-up questionnaire.

Questionnaire data

All participants who took part in the baseline examination completed a questionnaire regarding their lifestyle, medical and medication history. Socio-economic class and tobacco usage were defined as per Supplementary Methods, available in *Age and Ageing* online. Polypharmacy was defined as taking five or more regular medications [29]. Participants were asked if, at the time of completing the questionnaire, they experienced pain or discomfort (no/moderate/extreme).

Comorbidities

Prevalent (at/prior to baseline) and incident (between baseline and follow-up) cardiovascular disease were taken from doctor's diagnoses based on primary care records (from record reviews completed by participants' usual general practitioners). Prevalent diabetes mellitus was defined as either a physician-confirmed diagnosis of diabetes mellitus or a fasting serum glucose of greater than 7 mmol/l at baseline. To estimate multimorbidity, the total number of comorbidities (see Supplementary Methods are available in *Age and Ageing* online) were summed without weighing for each participant.

Cognitive testing

Cognitive skills were assessed using the Test Your Memory (TYM) instrument [30] and diagnoses of 'mild' or 'severe' cognitive impairment made; see Supplementary Methods, available in *Age and Ageing* online. TYM-based definitions in this cohort show similar cardiometabolic and sociodemographic associations to those seen in other studies with Alzheimer's dementia and mild cognitive impairment [31].

Physical examination

Blood pressure, body mass index (BMI), grip strength, walking speed and forced expiratory volume in 1 s were measured as described in Supplementary Methods, available in *Age and Ageing* online.

Electrocardiography

Twelve-lead electrocardiograms were recorded with a Siemens Sicard 460 instrument. Atrial fibrillation was diagnosed using the Minnesota Coding Scheme [32].

Blood measurements

Glucose, low-density lipoprotein, estimated glomerular filtration rate, CRP, IL-6, NT-proBNP and hs-cTnT were measured as described in Supplementary Methods, available in *Age and Ageing* online.

Prevalent and incident frailty

The Fried phenotype was used to define frailty [35]. Five variables were calculated at the baseline examination, three based on subjective self-report: unintentional weight loss (≥5% decrease in self-reported weight that was unintentional); exhaustion (answering 'no' to the question 'Do you feel full of energy?'); low physical activity (self-report of being less active or much less active than an average man); and two on objective measures: weakness (lowest fifth of grip strength distribution) and slow walking speed (lowest fifth of walking speed). Where measured walking speed was unavailable, self-report of low walking pace was used (self-report of walking speed, or being unable to walk more than a few steps, or < 200 yards, or difficulty walking across a room).

All living men were invited to complete a questionnaire 3 years later. Unintentional weight loss and low physical activity were calculated in the same way. Exhaustion was defined as answering 'often' to the questions: 'during the past week, how often did you feel that everything you did was an effort?' and/or 'During the past week, how often did you feel that you could not get "going"?" Slow walking speed was defined by self-report, as described above. Weakness was defined as self-report of 'fair' or 'poor' grip strength relative to men of the same age. This questionnaire-based determination of frailty status is as good a predictor of adverse outcomes (falls, disability and death) as the objectively-measured score in this cohort [36].

In all cases, men with three or more features were defined as 'frail', those with one or two as 'pre-frail', and those with none as 'robust'.

Inclusion and exclusion criteria

Men who were frail at baseline, or who had missing data on baseline frailty were excluded, as were those men missing baseline measurements of CRP; IL-6; hs-cTnT or NT-proBNP. Analyses were handled using complete-case analysis: participants with missing values for any variable used at that step of the analysis were excluded.

Statistical analysis

Analyses were performed using version 9.4 of the SAS System for Windows (Cary, NC, USA). Statistical significance was set at $P < 0.05$.

Descriptive statistics were used to report sample characteristics at baseline, with chi-square tests used for comparisons between groups for categorical variables; *t* tests were used for comparisons of normally distributed variables. Distributions for NT-proBNP, hsCRP, hs-TnT and IL-6 were positively skewed. Geometric means were calculated for these variables and comparisons made using the Kruskal–Wallis test.

Given the U-shaped relationship between BMI and frailty, [37] four BMI groups were calculated: <20; 20–24.9; 25–29.9 and ≥30, with the 20–24.9 group used as the reference.

NT-proBNP, hsCRP, hs-TnT and IL-6 values were divided into tertiles, with the bottom tertile used for each as the reference group.

Multivariate logistic regression was performed to examine the associations between the biomarkers of interest and incident frailty status. Frailty status at follow-up (frail versus robust/pre-frail) was used as the response variable, and NT-proBNP, hsCRP, hsTnT and IL-6 tertiles as categorical explanatory variables, adjusted also for age, prevalent myocardial infarction, heart failure or stroke at baseline and frailty status (robust or prefrail) at baseline. We then additionally adjusted for BMI group and other potential confounders linked to frailty and cardiovascular disease: low density lipoprotein, smoking status (never/stopped >10 years ago/stopped <10 years ago/current smoker), systolic blood pressure, use of antihypertensives (yes/no), use of statins (yes/no), prevalent diabetes mellitus (yes/no), FEV1, socioeconomic class (manual/non-manual/military), impaired cognition (normal/mild cognitive impairment/-severe cognitive impairment), pain (no pain/moderate or extreme pain), atrial fibrillation (yes/no) and total number of comorbidities (zero or one/two to four/five or more). Associations between baseline inflammation and/or cardiac biomarkers and incident frailty might be explained by the development of overt cardiovascular disease in the interim, so the development of incident myocardial infarction and/or heart failure and/or stroke between baseline and follow-up (yes/no) was also included as an explanatory variable.

To examine the trend across groups, these analyses were repeated with NT-proBNP, hsCRP, hsTnT and IL-6 tertiles modelled as continuous variables.

Supplementary analysis

Our study design is vulnerable to survivorship bias, as all four biomarkers are associated with mortality risk. [24,25,38,39]

We performed a supplementary multivariate logistic regression analyses, adjusting for age, and using NT-proBNP, hsCRP, hsTnT and IL-6 tertiles, as explanatory variables (each modelled separately), firstly with missing incident frailty data (yes/no) as the response variable, and then secondly with death prior to follow-up (yes/no), to determine if these were associated with missingness (for any reason) or mortality.

Results

Of the surviving cohort, 1,722 men (55%) attended the baseline examination. About, 100 men lacked baseline frailty data and were excluded, as were a further 303 men who were frail at baseline, leaving 1,319 robust or pre-frail men. 1,225 men had all four of hs-CRP, IL6, hs-TnT and NT-proBNP measurements at baseline and were used in the following analyses.

Follow-up incident frailty data were missing for 244 (20%) men; 66 (5.4%) men had died by the time of follow-up. The mean time between baseline and questionnaire completion was 2.9 years (SD 0.5 years).

Baseline characteristics

Table 1 shows the characteristics at baseline for the 981 men with follow-up data who did, and did not, develop frailty at a 3-year follow-up. About, 91 (9%) became frail. Details of the proportion of the cohort with each attribute of the frailty phenotype at baseline and at follow-up are given in Table 2. In bivariate analyses, men who developed frailty at follow-up were statistically more likely to be older, to have been pre-frail at baseline, to have had mild or severe cognitive impairment, to have moderate or severe pain, and to have multimorbidity, and were less likely to have been taking statins. Men who developed frailty tended to have lower diastolic blood pressure, lower FEV1 values and higher blood levels of hs-cTnT, NT-proBNP, CRP and IL-6. Men who were frail at follow-up were much more likely to have developed overt cardiovascular disease (stroke, heart failure or myocardial infarction) between baseline and follow-up.

Multivariate analyses

In multivariate analysis (Table 3), mutually adjusted for all four biomarkers, and adjusting for age, pre-frailty and prevalent stroke, myocardial infarction, and heart failure at baseline, statistically significant associations were seen between incident frailty and elevated IL-6 (second tertile OR 2.36, 95% CI 1.18–4.71, $P = 0.02$; third tertile OR 2.53, 95% CI 1.24–5.15, $P = 0.01$). Increased odds of frailty were seen with higher CRP (third tertile OR 1.87, 95% CI 0.97–3.57, $P = 0.06$) and hs-cTnT (third tertile 1.86, 95% CI 0.97–3.58, $P = 0.06$), but neither of these associations were statistically significant. Conversely, NT-proBNP appeared to show a U-shaped relationship, with a with decreased odds of frailty in those in the second tertile (OR 0.56, 95% CI 0.30–1.04, $P = 0.07$), though this was also not statistically significant.

When additionally adjusting for: socioeconomic class; BMI group; systolic blood pressure; smoking status; use of antihypertensives; use of statins; cognitive impairment; atrial fibrillation; polypharmacy; multimorbidity; pain; FEV1; low-density lipoprotein; eGFR and incident stroke, myocardial infarction and/or heart failure between baseline and follow-up, most associations were similar, with the top tertile of IL-6 retaining a statistically-significant association with incident frailty (third tertile OR 2.36, 95% CI 1.07–5.16, $P = 0.03$). However, the association between the third tertile of hs-cTnT and incident frailty became slightly stronger and gained statistical significance (third tertile OR 2.24, 95% CI 1.03–4.90, $P = 0.04$).

Missingness and mortality

In supplementary multivariate analyses (Table 4), adjusting for age, higher IL-6 (third tertile OR 1.89, 95% CI 1.29–2.79, $P = 0.001$), NT-proBNP (third tertile OR 1.56, 95% CI 1.01–2.23, $P = 0.02$) and hs-cTnT (third tertile OR 1.07, 95% CI 1.0–2.27, $P = 0.02$) levels were associated with statistically higher odds of missingness at follow-up. Higher levels of all four biomarkers were significantly associated with mortality prior to follow-up (CRP third tertile OR 2.08, 95% CI 1.12–3.84, $P = 0.02$, IL-6 third tertile OR 6.22, 95% CI 2.59–14.96, $P < 0.0001$, NT-proBNP third tertile OR 2.31, 95% CI 1.20–4.44, $P = 0.01$, hs-cTnT third tertile OR 3.79, 95% CI 1.68–8.58, $P = 0.001$). Similar significant associations were seen when biomarker tertiles were modelled continuously for trend.

Discussion

Summary

In this sample of community dwelling older men, elevated IL-6 was robustly associated with the development of frailty 3 years later. This association appears to be graded and independent of existing and incident clinically apparent cardiovascular disease, established cardiovascular risk factors, atrial fibrillation, renal impairment and lung disease (as measured by FEV1), polypharmacy, multimorbidity, pain, socioeconomic status and cognitive impairment. Elevated CRP, to a lesser extent, was associated with increased risk of incident frailty, but this was not statistically significant.

hs-cTnT and NT-proBNP showed differing associations with frailty risk. Higher hs-cTnT was associated with an increase in odds of frailty in the fully adjusted model, which was statistically significant. Conversely, moderately high NT-proBNP was associated with reduced odds of

Table 1. Baseline characteristics of selected sample of participants in the British Regional Heart Study aged 71–91 years in 2010–12 by incident frailty group

	Did not develop frailty (n = 890)	Developed frailty (n = 91)	P value
Age (years)	77.5 (4.2)	79.8 (4.8)	<0.0001
Longest-held occupation at entry: non-manual	490 (55%)	49 (54%)	0.99
Longest-held occupation at entry: manual	378 (43%)	39 (43%)	
Longest-held occupation at entry: military	20 (2%)	2 (2%)	
Frailty status at baseline			<0.0001
Robust	351 (39%)	7 (8%)	
Pre-frail	539 (61%)	84 (92%)	
Smoking status			0.48
Never smoked	361 (41%)	31 (34%)	
Long-term ex-smoker	468 (53%)	51 (56%)	
Recent ex-smoker	38 (4%)	5 (5%)	
Current smoker	22 (2%)	4 (4%)	
Comorbidities			
Previous myocardial infarction	106 (12%)	11 (12%)	0.96
Heart failure	31 (3%)	4 (4%)	0.65
Previous stroke	41 (5%)	8 (9%)	0.08
Diabetes mellitus	125 (14%)	18 (20%)	0.14
Taking five or more regular medications	301 (34%)	31 (34%)	0.96
Mild cognitive impairment	320 (38%)	45 (57%)	0.0003
Severe cognitive impairment	55 (7%) (missing cognitive data = 50)	9 (11%) (missing cognitive data = 11)	
Atrial fibrillation	60 (7%) (missing = 3)	9 (10%) (missing = 1)	0.25
Taking statin medication	433 (49%)	36 (40%)	0.10
Taking antihypertensive medication	457 (51%)	51 (56%)	0.39
No or one comorbidity	285 (32%)	10 (21%)	<0.0001
Two to four comorbidities	474 (53%)	43 (47%)	
Five or more comorbidities	131 (15%)	29 (32%)	
Experiencing moderate or extreme pain/discomfort at baseline	400 (45%)	54 (59%)	0.01
Developed new stroke, myocardial infarction and/or heart failure between baseline and follow-up	26 (3%)	11 (12%)	<0.0001
Physical measurements			
Body mass index group			0.11
BMI <20 kg/m^2	12 (1%)	4 (4%)	
BMI 20–24.9 kg/m^2	266 (30%)	22 (24%)	
BMI 25–29.9 kg/m^2	462 (52%)	47 (52%)	
BMI ≥30 kg/m^2	150 (17%)	18 (20%)	
Systolic blood pressure (mmHg)	146 (18) (missing = 1)	148 (20)	0.36
Diastolic blood pressure (mmHg)	77 (11) (missing = 1)	75 (12)	0.03
Forced expiratory volume in 1 s (l)	2.54 (0.5) (missing = 45)	2.25 (0.6) (missing = 10)	<0.0001
Biomarkers			
Estimated glomerular filtration rate (ml/min/1.73 m^2)	74.7 (16) (missing = 1)	73.7 (21) (missing = 0)	0.60
hs-cTnT (pg/ml)	9.4 (6.5–14)	12.7 (9.5–18)	<0.0001
First tertile hs-cTnT (<7.8 pg/ml)	327 (37%)	18 (20%)	
Second tertile hs-cTnT (7.8–13.28 pg/ml)	302 (34%)	29 (32%)	
Third tertile hs-cTnT (≥13.3 pg/ml)	261 (29%)	44 (48%)	
NT-proBNP (pg/ml)	112 (61–224)	150 (58–381)	0.0197
First tertile NT-proBNP (<81 pg/ml)	315 (35%)	27 (30%)	
Second tertile NT-proBNP (81–197 pg/ml)	311 (35%)	22 (24%)	
Third tertile NT-proBNP (≥198 pg/ml)	264 (30%)	42 (46%)	
CRP (mg/l)	1.1 (0.6–2.4)	2.0 (0.9–4.4)	<0.0001
First tertile CRP (<0.79 mg/l)	318 (36%)	16 (18%)	
Second tertile CRP (0.79–2.02 mg/l)	302 (34%)	33 (36%)	
Third tertile CRP (≥2.03 mg/l)	270 (30%)	42 (46%)	
IL-6 (pg/ml)	2.7 (1.7–3.9)	3.5 (2.4–5.4)	<0.0001
First tertile IL-6 (<2 pg/ml)	334 (38%)	12 (13%)	
Second tertile IL-6 (2–3.61 pg/ml)	304 (34%)	38 (42%)	
Third tertile IL-6 (≥3.62 pg/ml)	252 (28%)	41 (45%)	

For normally distributed continuous variables, values are mean (SD) and P values for t tests; for hs-TnT, NT-proBNP, CRP and IL-6, values are geometric mean (interquartile range) and P values for Kruskall–Wallis tests; for categorical variables values are n (% of total by column) and P values for Chi-square tests. Missing values are indicated if present.

Table 2. Proportion of cohort at baseline and follow-up with each attribute of the frailty phenotype

	Baseline (n = 1,225)	At 3 year follow-up (n = 981)
Exhaustion	548 (45%)	83 (9%)
Unintentional weight loss	81 (7%)	108 (11%)
Low physical activity	240 (20%)	199 (20%)
Slow walking speed	110 (9%)	215 (23%)
Low grip strength	189 (15%)	156 (16%)

incident frailty, though this was not statistically significant, and high NT-proBNP showed no association with incident frailty.

Inflammation and frailty

To our knowledge, this is the first longitudinal study of community-dwelling adults to report a robust association between an inflammatory marker (IL-6) and the subsequent development of frailty, supporting the argument that inflammation plays a causal role in the frailty syndrome. This association may be affected by selection and survival bias, as men with higher IL-6 levels were more likely to miss follow-up, and men with higher IL-6 or CRP levels were more likely to die before follow-up. Given that frailty increases mortality risk, it is plausible that decedents were more likely to become frail prior to death; if so, the associations between IL-6, CRP and frailty shown here may be underestimates.

The association between IL-6 and incident frailty is consistent with the strong cross-sectional associations reported in numerous samples between inflammation and frailty, [9] but contrasts with the four extant longitudinal studies, which found no association between IL-6 and frailty. In two of those, the assays used were relatively insensitive, meaning that serum IL-6 levels were undetectable in 47% [17] and 89% [16] of their samples, which significantly limited their ability to stratify participants with low levels of IL-6. Two others analysed IL-6 and frailty in a much smaller subsample (n = 151 and n = 311 vs. n = 895 in this study) and may have been under-powered to detect this association [15,18].

The associations between CRP and frailty in our study did not reach statistical significance, although this was borderline and an association cannot be fully discounted. We may have lacked power to detect a statistically significant effect. The lack of a significant association does, however, accord with prior findings of null or limited subgroup associations between CRP and incident frailty. IL-6 is upstream of CRP in the inflammatory cascade and has more widespread effects, which—in the setting of inflammation—include the production of CRP and other acute phase proteins, neutrophil production, lipolysis in the liver and proteolysis in muscle. IL-6 also regulates multiple other physiological processes, including tissue maintenance, tissue repair, energy metabolism and, in some cases, may counteract the inflammatory cascade [40]. IL-6 might reflect a pro-inflammatory state that leads to frailty, [41] but may also represent a response to conserve and mobilise energy in the face of other stressors, which may contribute to the energy dysregulation seen in frailty [42]. In our study, the association between IL-6 and frailty persisted despite adjustment for a range of comorbidities, including incident cardiovascular disease between baseline and follow-up, suggesting that these do not fully explain the relationship between inflammation and frailty.

Cardiac biomarkers and frailty

We observed a two-fold increase in the odds of incident frailty in the highest group of hs-cTnT and incident frailty in the fully adjusted analysis. However, there were no statistically significant associations between frailty and hs-cTnT in the initial analysis. From the initial to the fully adjusted analysis, n reduced from 981 to 895, mostly due to the loss of men with missing FEV1 values and missing assessments of cognition. This may have introduced bias via non-random missingness (higher risk men may have been less able to perform lung function testing or cognitive testing and therefore more likely to be missing) and led to a type I error. However, we also found evidence of survivorship bias: elevated hs-cTnT was associated with increased odds of missingness at, and mortality prior to, follow-up. As above, if men who died were more likely to have become frail prior to death, this may have weakened the strength of the association with incident frailty. hs-cTnT levels in older people are associated with the presence of comorbidity [43], which could be a residual confounder, though the association between hs-cTnT and incident frailty seen here was not weakened by adjustment for multimorbidity. Alternatively, myocardial injury may be related to the development of frailty.

Unexpectedly, given prior reports of positive cross-sectional association between NT-proBNP and frailty, [22] we found a suggestion of a U-shaped relationship, with men with NT-proBNP levels between 81–197 pg/ml having lower odds of incident frailty than those with levels below 81 pg/ml, though these associations did not reach statistical significance. NT-proBNP levels are inversely related with type 2 diabetes mellitus risk [44], which itself is linked with frailty [45]. Those with moderately high NT-proBNP might have been less likely to develop type 2 diabetes mellitus and frailty. Regardless, the data here do not support an extension of the cross-sectional positive relationship between NT-proBNP and frailty: higher NT-proBNP does not appear to be associated with elevated risk of frailty in this sample. Again, however, elevated NT-proBNP was associated with greater odds of missingness and mortality, which is likely to have introduced survivorship bias.

Table 3. Associations between biomarkers and incident frailty in multivariate logistic regression analyses. Statistically significant ($P < 0.05$) associations are bolded

		Odds ratio for incident frailty	95% confidence interval for OR	*P* value
Mutually adjusted and also for age, pre-frailty, prevalent myocardial infarction, heart failure and stroke (*n* = 981)	First CRP tertile	1	–	–
	Second CRP tertile	1.68	0.87–3.22	0.11
	Third CRP tertile	1.87	0.97–3.57	0.06
	CRP trend	*1.31*	*0.97–1.78*	*0.08*
	First IL-6 tertile	1	–	–
	Second IL-6 tertile	**2.36**	**1.18–4.71**	**0.02**
	Third IL-6 tertile	**2.53**	**1.24–5.15**	**0.01**
	IL-6 trend	***1.48***	***1.08–2.02***	***0.02***
	First NT-proBNP tertile	1	–	–
	Second NT-proBNP tertile	0.56	0.30–1.04	0.07
	Third NT-proBNP tertile	0.92	0.51–1.67	0.79
	NT-proBNP trend	*1.00*	*0.73–1.36*	*0.99*
	First hs-cTnT tertile	1	–	–
	Second hs-cTnT tertile	1.33	0.70–2.52	0.38
	Third hs-cTnT tertile	1.86	0.97–3.58	0.06
	hs-cTnT trend	*1.39*	*1.00–1.91*	*0.05*
Additionally adjusted for incident MI/HF/stroke, socioeconomic class, BMI class, systolic blood pressure, smoking status, use of antihypertensives, use of statins, cognitive impairment, atrial fibrillation, polypharmacy, multimorbidity, pain, FEV1, low-density lipoprotein, eGFR (*n* = 895)	First CRP tertile	1	–	–
	Second CRP tertile	2.05	0.95–4.40	0.07
	Third CRP tertile	1.83	0.84–4.00	0.12
	CRP trend	*1.24*	*0.86–1.79*	*0.24*
	First IL-6 tertile	1		
	Second IL-6 tertile	2.11	0.99–4.48	0.05
	Third IL-6 tertile	**2.36**	**1.07–5.17**	**0.03**
	IL-6 trend	*1.45*	*1.00–2.09*	*0.05*
	First NT-proBNP tertile	1	–	–
	Second NT-proBNP tertile	0.48	0.23–1.01	0.05
	Third NT-proBNP tertile	0.87	0.42–1.78	0.70
	NT-proBNP trend	*0.96*	*0.66–1.39*	*0.82*
	First hs-cTnT tertile	1	–	–
	Second hs-cTnT tertile	1.67	0.80–3.47	0.17
	Third hs-cTnT tertile	**2.24**	**1.03–4.90**	**0.04**
	hs-cTnT trend	*1.43*	*0.98–2.08*	*0.06*

Bold: Statistically significant at $P < 0.05$. Italics: Modelled with tertiles as continuous explanatory variable to demonstrate trend across groups.

Strengths and limitations

This study is the first to analyse the role of cardiac biomarkers alongside inflammatory markers in incident frailty. It benefits from a reasonably large sample size and adjustment for multiple possible confounders, including cognition, polypharmacy and multimorbidity. However, it is possible that residual confounding remains. We used a simple unweighted numerical scale for total comorbidities when accounting for multimorbidity, as we lacked the exact data to compute a previously validated weighted index. Our approach does not account for the severity of each comorbidity and may not fully reflect the degree of comorbidity burden. Our non-cardiovascular comorbidity data was mostly based on self-report, which may lack validity.

Our frailty assessment is easily replicable in other settings, including clinical practice. However, part of the initial score, and the entire follow-up measure, is based on subjective self-report, which may suffer from reporting bias. Our definition of 'exhaustion' was more stringent at follow-up than at baseline (as reflected by the reduced prevalence after 3 years), and this may have led to an over-estimate of frailty state at baseline and—relative to this—an under-estimate of frailty status at follow-up, which may have reduced the number of incident frailty cases we detected. The cohort is entirely male and almost entirely of White European origin, which may limit generalisability of findings. One-fifth of the original baseline cohort was lost to follow-up. Our results may be affected by survivorship bias, as discussed above, and by non-random missing data.

Implications for future research

Our reported association between IL-6 and frailty should be replicated in other cohorts with similar methodology. Validated comorbidity indices could be used to further reduce the potential for confounding by multimorbidity. Further larger longitudinal analyses of incident frailty, NT-proBNP and hs-cTnT should be performed, which may confirm or refute the possibility of associations raised here. Survivorship bias could be mitigated by frequent re-assessments of frailty status; as this information is increasingly being recorded in routine clinical practice, this could be obtained from those sources.

Table 4. Age-adjusted associations between missingness at follow-up, mortality at follow-up and biomarkers

		Missingness at follow-up (n = 244 missing cases)			Mortality at follow-up (n = 66 deaths)		
		Odds ratio	95% confidence interval of OR	P value	Odds ratio	95% confidence interval of OR	P value
CRP	First tertile	1			1		
	Second tertile	0.99	0.39–1.41	0.94	0.87	0.42–1.79	0.71
	Third tertile	1.33	0.95–1.88	0.10	**2.08**	**1.12–3.84**	**0.02**
	Trend	*1.16*	*0.98–1.38*	*0.09*	***1.53***	***1.11–2.11***	***0.01***
IL-6	First tertile	1			**1**		
	Second tertile	1.11	0.76–1.63	0.58	**3.06**	**1.21–7.74**	**0.02**
	Third tertile	**2.14**	**1.50–3.05**	**<0.0001**	**6.22**	**2.59–14.96**	**0.0001**
	Trend	***1.50***	***1.25–1.80***	***<0.0001***	***2.34***	***1.62–3.37***	***<0.0001***
NT-proBNP	First tertile	1			1		
	Second tertile	1.09	0.76–1.58	0.64	0.91	0.43–1.94	0.80
	Third tertile	**1.56**	**1.01–2.23**	**0.02**	**2.31**	**1.20–4.44**	**0.01**
	Trend	***1.26***	***1.05–1.51***	***0.01***	***1.64***	***1.16–2.30***	***0.005***
hs-cTnT	First tertile	1			1		
	Second tertile	1.17	0.81–1.70	0.40	**2.57**	**1.12–5.89**	**0.03**
	Third tertile	**1.56**	**1.07–2.27**	**0.02**	**3.79**	**1.68–8.58**	**0.001**
	Trend	***1.25***	***1.04–1.51***	***0.02***	***1.82***	***1.27–2.61***	***0.001***

Bold: Significant at $p < 0.05$. Italics: Continuous modeling of biomarker tertiles for trend.

Supplementary Data: Supplementary data mentioned in the text are available to subscribers in *Age and Ageing* online.

Declaration of Conflicts of Interest: None. A full list of DGJM's interests can be found at http://www.whopaysthisdoctor.org/doctor/500/active.

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