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CLINICAL RESEARCH
Prevention and epiemiology

Biomarkers of inflammation predict both vascular and non-vascular mortality in older men

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Aims To compare the predictive value of inflammatory biomarkers and lipids for vascular and non-vascular mortality in older men.

Methods and results The relevance of inflammatory biomarkers and lipids for vascular and non-vascular mortality was assessed in a prospective study of 5360 men (mean age 77 years) followed for 7 years. Vascular mortality was positively associated with log C-reactive protein (lnCRP), fibrinogen and total/HDL-C (high-density lipoprotein cholesterol), and inversely associated with albumin [age adjusted hazard ratio (HR) per 2-SD higher usual level (approximately the difference between the top and the bottom thirds of the distribution): 2.09 for lnCRP; 1.70 for fibrinogen; 0.50 for albumin and 1.45 for total/HDL-C]. The associations with the inflammatory markers were attenuated after adjustment for established risk factors, including lipids [adjusted HRs: 1.86 (lnCRP); 1.44 (fibrinogen); 0.51 (albumin)], and further attenuated (and, for fibrinogen, no longer predictive) after adjustment for each other [fully adjusted HRs: 1.60 (lnCRP); 1.01 (fibrinogen); 0.61 (albumin)]. Higher CRP and lower albumin levels were also associated with significantly raised non-vascular mortality independently of other characteristics [fully adjusted HRs: 1.62 (lnCRP); 0.65 (albumin)].

Conclusion In this cohort of older men, higher CRP and lower albumin levels strongly predicted both vascular and non-vascular mortality, independently of other characteristics.

Keywords C-reactive protein • Lipids • Prediction of mortality in old age

Introduction

Atherosclerosis involves a chronic inflammatory component, comprising an interaction of immune mechanisms with metabolic risk factors that manifests as vascular events.[1–3] Plasma levels of circulating biomarkers of inflammation are believed to reflect the severity of inflammation and extent of underlying atherosclerosis.[4] C-reactive protein (CRP) – the acute phase protein synthesized primarily by the liver that is stable and readily measured – is currently the most widely used biomarker of inflammation.[3] However, fibrinogen (a major protein involved in coagulation) and albumin (the most abundant protein in plasma) are also used as biomarkers for thrombosis and general health, respectively.[3,4] The American Heart Association Guidelines on the use of biomarkers for vascular risk prediction advocate that an individual with plasma levels of CRP $\geq$ 3 mg/L on more than two occasions in the absence of prior vascular disease should be targeted for more intensive therapy for prevention of vascular disease.[3]

While associations of CRP, fibrinogen, and albumin with vascular diseases have been extensively studied in middle-aged individuals.[5–13] These biomarkers are highly correlated with each other and with other known vascular risk factors, and it is not known if they merely reflect the severity of the underlying atherosclerosis or are actually involved in its pathogenesis. A meta-analysis of prospective cohort studies of middle-aged individuals reported that the strength of the association of CRP with ischaemic heart

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disease (IHD) was less than previously believed, after taking account of the known vascular risk factors, such as smoking, blood pressure, blood lipids, and diabetes mellitus.[10] Moreover, a meta-analysis of cohort studies of fibrinogen and mortality also demonstrated a marked attenuation in the strength of the associations of fibrinogen with vascular and non-vascular mortality after taking account of baseline values of known vascular risk factors measured in middle age.[14] However, there is only limited evidence about the relevance of these biomarkers for prediction of both vascular and non-vascular mortality when measured in older people.[15–18] Since mean plasma levels of both low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) decline with age and onset of disease, it is possible that biomarkers of inflammation may be more strongly related to vascular risk in older people than measures of blood lipids.

The aims of this study were: (i) to compare the shape and strength of the associations of vascular mortality with biomarkers of inflammation (CRP, fibrinogen and albumin) and with blood lipids (total/HDL-cholesterol ratio, previously shown in this population to be strongly predictive of IHD mortality[19]) in men with and without a history of prior chronic disease (cardiovascular, diabetes or cancer); (ii) to assess the effects on these associations of adjustment for known vascular risk factors; (iii) to compare the associations with vascular mortality to those observed with non-vascular mortality; and (iv) to estimate the predictive value of elevated levels of CRP ($\geq$3 mg/L) for overall survival, in men with and without a history of prior chronic disease.

Methods

Study population

Nineteen thousand and nineteen male civil servants working in London and aged 40–69 years were recruited between 1967 and 1970 into the Whitehall study, details of which have been previously reported.[20,21] Following the success of a pilot study of the feasibility of re-contacting surviving participants in 1995, all 8448 surviving participants were sought for re-survey in 1997–1998.[22] A postal questionnaire asked details of diagnoses (e.g. ever told by a doctor that they had had angina, heart attack or diabetes), medications taken in the last month, smoking status, and last known civil service employment grade.[23] The 7044 participants who responded to the re-survey (83%) were subsequently sent a blood collection kit and asked to attend their local surgery to have a blood sample collected and measurements of blood pressure, height and weight recorded. Non-fasting blood samples were obtained from 5434 men (77% of respondents), from which blood lipids (total and HDL cholesterol) and measurements of inflammatory markers were available for 5364 men (98.7%). Medical history, medication use, and mortality follow-up status (discussed later) were recorded for 5360 of these men (99.9%). The non-respondents in 1997 were older and had a lower employment grade compared with the respondents, but the response rates were not associated with total cholesterol levels recorded in 1967–1970 (23). The re-survey was approved by the ethics committees of the participating institutions.

Laboratory methods

Blood was collected into a 10 mL vacutainer containing potassium EDTA with 0.34 mmol/L aprotinin. These whole blood samples were mailed in sealed transport tubes at room temperature to the laboratory in Oxford. The mean time in the post was 1.3 days (range 0–7 days), with 78% arriving within 24 h and 96% arriving within 48 h of blood collection. On arrival in the laboratory, the blood was centrifuged, and the plasma was aliquoted for storage at -40°C. All lipid analyses were performed on Beckman Synchron CX4 and CX5 auto-analysers (Beckman Coulter UK Limited, High Wycombe, UK), which were programmed to subtract a sample blank absorbance reading from the final reaction absorbance to correct for any interference from haemolysis, which can arise when samples are transported for a prolonged period. Total cholesterol was measured enzymatically using Beckman reagent and HDL-C was measured directly using N-geneous reagents (Bio-Stat Limited, Stockport, UK). Details of the methods used to measure the other lipid indices [apolipoprotein A_1 (Apo A_1), apolipoprotein B (Apo B) and directly measured LDL-C] have been published.[19] CRP, fibrinogen, and albumin were analysed using Dade-Behring reagents on a Dade-Behring Nephelometer II analyzer (Dade-Behring, Marborg, Germany). The intra-assay coefficients of variation, based on repeat analysis of laboratory control material, were 2% for total cholesterol, 5% for HDL-C, and 3–4% for CRP, fibrinogen, and albumin. Previous studies had indicated that minor changes in blood lipids and inflammatory markers arose due to delayed separation of mailed blood samples: so, where possible (>99% of samples), blood lipid, and inflammatory marker values were adjusted for duration of time spent in the post before separation of plasma;[24] and also for date of assay to avoid assay drift.

Mortality follow-up

Participants were flagged for mortality at the Office for National Statistics, which provided the date and cause [including International Classification of Disease (ICD) codes] of all deaths occurring until the end of September 2005. The mean follow-up period was 6.8 years (maximum 8.4 years). Cause-specific mortality was coded using ICD-9 up to August 2002 and ICD-10 subsequently. IHD deaths were pre-defined as those allocated ICD-9 codes 410–414 and ICD-10 codes I20–I25 as the underlying cause of death. Vascular deaths (heart disease, stroke and other vascular disease) were so defined if coded as ICD-9 codes 390–459, 798 or ICD-10 codes I00–I99, R96, with all other causes of death being defined as non-vascular.

Statistical methods

Hazard ratios and floating absolute risks

The associations between mortality risk and the different biomarkers were assessed using Cox proportional hazards regression by time since blood collection, initially after adjustment only for age, and subsequently after adjustment for age and other characteristics. Analyses were performed in all men, and separately in men with and without a history of prior disease (defined as myocardial infarction, angina, stroke, diabetes or non-skin cancer). [Tests for differences in hazard ratios (HRs) between men with and men without prior disease were performed by including into regression models interaction terms between the exposure of interest and prior disease, and such tests were considered statistically significant if the two-sided *P*-value was <0.05.] To assess the approximate shape of the risk associations with each blood marker, HRs and 95% confidence intervals (CI) were estimated for each quarter of the blood marker distributions and plotted

against 'usual', rather than measured, values (see *'Correction for regression dilution bias'* discussed later). These HRs are presented as 'floating absolute risks', which do not alter their values but merely ascribes an approximate 95% CI to the HR in every group (including even the reference group with HR 1.0).[25]

The log-linearity assumption was assessed by including a quadratic term into the model, while the proportional hazards assumption was assessed by including an interaction term between the exposure and the logarithm of the time to event (given the number of tests, these were considered statistically significant if the two-sided *P*-value was <0.01). Age-adjusted estimates of SD were derived from all men, and rescaled to correspond to 'usual', rather than measured, values (given later). Subsequently, HRs associated with 2 standard deviation (SD) differences in measured levels were estimated. A 2-SD difference was chosen because it is approximately equivalent to the mean difference between the top and bottom thirds of a normal distribution. (Since the baseline distribution of CRP was not normally distributed, log CRP was used in analyses rather than CRP). To assess the extent that any identified associations could be due to confounding, analyses relating each inflammatory marker to risk were repeated after adjustment for: (a) known 'classical' vascular risk factors other than lipids [cigarette smoking status (current/ex/never), drinking status (current/non), body mass index, the average of the measured systolic and diastolic blood pressure, recall of a diagnosis of hypertension, and medication use (antiplatelet drugs, statins or blood pressure lowering drugs)]; (b) employment grade at baseline; (c) lipids [HDL-C, total cholesterol, apolipoprotein A_1 (Apo A_1), Apo B (apolipoprotein B), ratio of LDL-C to Apo B and ratio of HDL-C to Apo A_1]; and (d) the other inflammatory markers measured. The change in deviance upon inclusion of each biomarker into these 'adjusted' models was used to provide a quantitative indication of the extent that the risk-relationships were due to confounding (all analyses were based on the same sample size, with any missing data being imputed as necessary).

Correction for regression dilution bias

Due to the combined effects of measurement error and within-person variability, single baseline measurements of biomarkers do not, on average, reflect the '*usual*' medium term levels,[26] leading to underestimation of the importance of the *usual* levels of biomarkers to risk when baseline levels are used in analyses. HRs were therefore corrected for this 'regression dilution bias' by dividing the log HR associated with baseline levels (and its standard error) by *r*, the correlation coefficient of the biomarker values measured in individuals at different times. (Similarly, the 'usual SD' of each blood marker was obtained from the measured SD through multiplication by $\sqrt{r}$). The correlation coefficients between repeat blood marker values were obtained from samples collected on two occasions at an interval of 2–3 years from 1044 healthy middle-aged participants in a separate study, which used identical methods for blood collection and lipid analysis in the same laboratory over the same period[26] (and previous reports have shown that such correlation coefficients are largely independent of age and sex[26]). The correlation coefficients used to correct the HRs for regression dilution bias were 0.60 for log CRP, 0.59 for fibrinogen, 0.46 for albumin, and 0.74 for the ratio of total to HDL-C.[27]

Estimated survival from age 70 years by C-reactive protein level and prior disease

Survival from age 70 onwards was estimated in each of four strata: (i) men without prior disease and low (<3 mg/L) CRP; (ii) men without prior disease and high (≥ 3 mg/L) CRP; (iii) men with prior disease and low CRP; and (iv) men with prior disease and high CRP. For each stratum, observed death rates in each of five-year age ranges (from 70–75 years to 90–95 years, excluding the small numbers of deaths outside this age range) were calculated, and an inverse variance weighted linear regression of the logarithm of the death rates on the mean age within each age period was performed. This allowed for estimation of the probability of surviving any given year of age conditional on being alive at the start of that year. Consecutive multiplication of these survival probabilities using a probability of 1 at age 70 (i.e. only considering risk from age 70 onwards) yielded a predicted survival curve based on observed death rates for each stratum.

Results

Characteristics of study participants

Table 1 shows selected characteristics of all 5360 men, the 3541 men (66%) without prior cardiovascular disease, diabetes mellitus or cancer, and the 1819 men (34%) with prior disease. The mean age at resurvey was 76.9 (range 66–96) years, and was 1 year higher among men with prior disease (77.6 years) than those without prior disease (76.5 years). Previous myocardial infarction was reported by 11.3% of all men, angina by 14.2%, stroke by 7.1%, diabetes by 5.9%, and cancer by 7.8%. Approximately one-third of all men (one-half with prior disease and one-fifth without) were taking antiplatelet drugs, with very similar estimates for blood pressure lowering drugs. Use of statins at the time of this survey in 1997 was low (2.3% overall). The majority of men were non-smokers (87.2%) and current drinkers (77.8%), mean blood pressure was 145/80 mmHg, and mean body mass index 25.2 kg/m^2. Over 80% of men were of a high socio-economic class (as determined by last known employment grade). Men with prior disease had, on an average, higher baseline total/HDL-C ratio, CRP and fibrinogen levels, and lower albumin levels, than men without prior disease (all $P < 0.0001$), although the clinical differences in mean levels were mostly small.

Associations with ischaemic heart disease and all vascular mortality

Among all 5360 men, 853 died from vascular disease within the following 7 years, including 446 from IHD [vascular death rate: 23.4 per 1000 person years (py); IHD death rate: 12.2 per 1000 py]. In the subset of 3541 men with no prior disease, 422 (16.6 per 1000 py) died from vascular disease, including 192 (7.5 per 1000 py) from IHD; while in the 1819 men with prior disease, 431 (38.9 per 1000 py) died from vascular disease, including 254 (22.9 per 1000 py) from IHD. *Table 2* shows the age adjusted HRs associated with 2-SD differences in *usual* levels of each

Table 1 Selected characteristics of all participants and men with and without prior disease at the time of blood collection

Baseline characteristics	All men (n = 5360)	No prior disease (n = 3541)	Prior disease[a] (n = 1819)
Mean (SD) age, years	76.9 (4.9)	76.5 (4.8)	77.6 (5.0)
Medical history, *n* (%)			
Myocardial infarction	606 (11.3)	-	606 (33.3)
Angina	760 (14.2)	-	760 (41.8)
Stroke	383 (7.1)	-	383 (21.1)
Diabetes mellitus	315 (5.9)	-	315 (17.3)
Cancer	420 (7.8)	-	420 (23.1)
Hypertension	1697 (31.7)	954 (26.9)	743 (40.8)
Medication, *n* (%)			
Anti-platelet drugs	1768 (33.0)	797 (22.2)	981 (53.9)
Blood pressure lowering drugs	1713 (32.0)	831 (23.5)	882 (48.5)
Statins	125 (2.3)	24 (0.7)	101 (5.6)
Lifestyle, *n* (%)			
Current smoker	684 (12.8)	499 (14.1)	185 (10.2)
Current drinker	4157 (77.8)	2790 (79.1)	1367 (75.4)
Physical measurements, mean (SD)			
Systolic BP, mmHg	145 (20)	145 (20)	144 (21)
Diastolic BP, mmHg	80 (11)	81 (11)	79 (11)
Body mass index, kg/m^2	25.2 (3.2)	25.2 (3.2)	25.4 (3.3)
Socio-economic grade			
Clerical/manual grade	930 (17.4)	624 (17.7)	306 (16.8)
Higher grade	4422 (82.6)	2910 (82.3)	1512 (83.2)
Laboratory data, mean (SD)			
lnCRP, ln mg/L	0.59 (1.11)	0.51 (1.10)	0.76 (1.11)
Fibrinogen, g/L	3.54 (0.85)	3.50 (0.82)	3.63 (0.89)
Albumin, g/L	39.7 (2.90)	39.88 (2.77)	39.34 (3.10)
Total/HDL ratio	5.71 (2.59)	5.58 (2.46)	5.95 (2.82)

[a]Prior disease defined as prior cardiovascular disease (i.e. diagnosis of angina, myocardial infarction or stroke), diabetes mellitus or (non-skin) cancer.

blood marker in all men, and in the subsets with and without prior disease for IHD and for all vascular mortality. *Figure 1* shows the associations graphically for each quarter of the marker distributions for vascular mortality.

Overall, there were strong positive associations of IHD and of all vascular mortality, for log CRP, fibrinogen and total/HDL-C, and a strong inverse association for albumin. These associations were approximately log-linear throughout the ranges of values studied, with the exception of albumin and IHD mortality, in which the magnitude of the risk relationship was greater at lower albumin levels ($P = 0.0004$ for the quadratic component). For albumin, the risk-relationships with IHD and all vascular mortality also appeared to be more pronounced during the early compared with later years of follow-up ($P = 0.01$ for both tests of proportionality). The average strength of the associations with IHD and with all vascular mortality for each of the biomarkers were similar in men with and without prior disease (*Table 2*), again with the exception of albumin and IHD mortality, in which a strong inverse relationship was observed among those with prior disease and no apparent relationship among those without prior disease. For all vascular mortality, however, CRP and albumin were equally strong predictors (albeit in the opposite direction) in both men with and without prior disease, and were stronger predictors than either total/HDL-C ratio or, to a lesser degree, fibrinogen (indicating that the apparent interaction between albumin and prior disease for IHD mortality was probably due to chance).

Associations with non-vascular mortality

One thousand one hundred and six men died from non-vascular causes (30.3 per 1000 py), including 605 men without prior disease (23.8 per 1000 py) and 501 men with prior disease (45.2 per 1000 py). *Figure S1* (see Supplementary material online) shows that CRP and albumin displayed similarly strong (but

Table 2 Comparison of the predictive strength of the inflammatory markers and total/HDL-C (high-density lipoprotein cholesterol) ratio for ischaemic heart disease (IHD) mortality ($n = 446$), all vascular mortality ($n = 853$) and non-vascular mortality ($n = 1106$) in (i) all men, (ii) men with no prior disease and (iii) men with prior disease

	Hazard ratio (95% confidence interval) per 2-standard deviation higher usual level (i.e. ~top vs. bottom third)			Prior vs. No prior disease, P^a
	All men ($n = 5360$)	No prior disease ($n = 3541$)	Prior disease ($n = 1819$)	
IHD mortality				
lnCRP	2.52 (2.0, 3.17)	2.43 (1.71, 3.45)	2.31 (1.69, 3.14)	0.88
Fibrinogen	1.79 (1.42, 2.26)	2.07 (1.44, 2.96)	1.42 (1.04, 1.95)	0.15
Albumin	0.57 (0.43, 0.74)	0.92 (0.59, 1.44)	0.47 (0.34, 0.65)	0.02
Total/HDL-C	1.52 (1.28, 1.80)	1.68 (1.26, 2.25)	1.25 (1.00, 1.57)	0.16
Vascular mortality				
lnCRP	2.09 (1.77, 2.48)	1.92 (1.51, 2.44)	2.05 (1.61, 2.61)	0.79
Fibrinogen	1.70 (1.43, 2.02)	1.74 (1.35, 2.24)	1.50 (1.18, 1.90)	0.43
Albumin	0.50 (0.42, 0.61)	0.60 (0.45, 0.80)	0.48 (0.37, 0.61)	0.49
Total/HDL-C	1.45 (1.27, 1.65)	1.48 (1.19, 1.84)	1.28 (1.08, 1.52)	0.52
Non-vascular mortality				
lnCRP	2.00 (1.73, 2.33)	1.63 (1.33, 1.99)	2.38 (1.91, 2.97)	0.01
Fibrinogen	1.65 (1.41, 1.92)	1.40 (1.13, 1.74)	1.79 (1.45, 2.21)	0.11
Albumin	0.44 (0.37, 0.52)	0.53 (0.42, 0.68)	0.40 (0.32, 0.50)	0.12
Total/HDL-C	0.95 (0.81, 1.10)	0.78 (0.62, 0.99)	0.99 (0.81, 1.20)	0.11

All analyses are adjusted for age only. The correlation coefficients used to correct the hazard ratios (HRs) for regression dilution bias were estimated over a 2-year period among controls in the ISIS case-control study. The correlation coefficients were 0.60 for lnCRP, 0.59 for fibrinogen, 0.46 for albumin, and 0.74 for the ratio of total to HDL cholesterol.[27] The HRs among all men need not necessarily lie between the separate estimates for men with and men without prior disease because the analyses in all men do not adjust for prior disease.

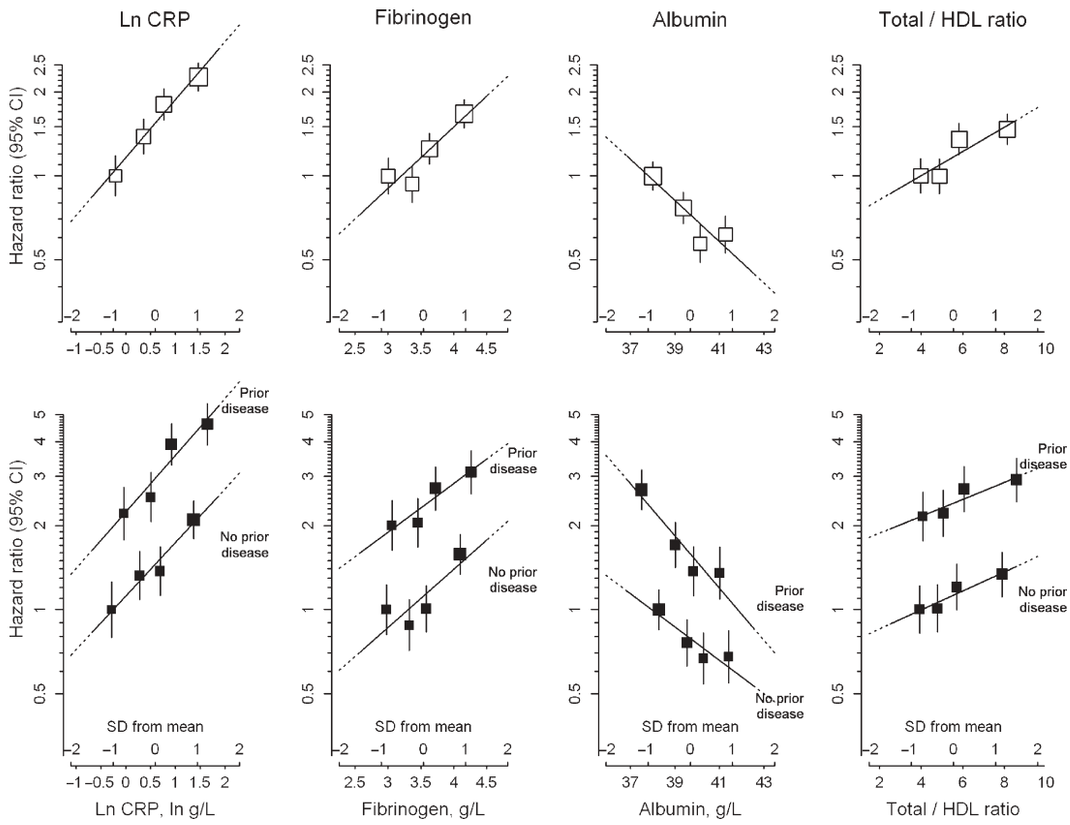
[a]Test for difference in the HR observed in men with prior disease and that observed in men without prior disease.

opposite) associations with non-vascular mortality, and fibrinogen displayed a positive, though somewhat weaker, association. Evidence of non-linearity in these risk-associations was observed: the relevance of CRP and fibrinogen to non-vascular mortality risk was greater at higher exposure levels, while the relevance of albumin to non-vascular mortality risk was greater at lower exposure levels. Each of the inflammatory markers also appeared to exhibit stronger risk relations in the earlier years of follow-up compared with the later years ($P < 0.001$ for each test of proportionality). *Table 2* shows the average strength of the associations observed between each of the markers and non-vascular mortality (i.e. over the range of values studied and the period of follow-up). For CRP, fibrinogen, and albumin, the strength of the associations with non-vascular mortality closely mirrored those observed for vascular mortality. Risk associations were generally stronger among men with a history of prior disease (significantly so for CRP) compared with men without such a history. The ratio of total to HDL-C displayed no overall association with non-vascular mortality (though a marginally significant inverse association was observed among the subset without prior disease).

Independence of known vascular risk factors

The associations of plasma levels of CRP, fibrinogen, and albumin with each other and with blood lipids (LDL-C and HDL-C, Apo B, and Apo A_1) are shown in *Figure S2*(see Supplementary material online). CRP was weakly correlated with LDL-C ($r = 0.01$), Apo B ($r = 0.07$) and the ratio of LDL-C to Apo B ($r = -0.10$), but was moderately related with HDL-C ($r = -0.22$), Apo A_1 ($r = -0.19$) and the ratio of HDL-C/Apo A_1 ($r = -0.21$). Correlations between fibrinogen and the different lipid indices were of a similar magnitude. Albumin levels were modestly correlated with LDL-C ($r = 0.14$) and Apo B ($r = 0.17$), but not with HDL-C ($r = 0.05$), ApoA_1 ($r = 0.13$), or the lipid ratios ($r = -0.05$ and $r = 0.00$, respectively for LDL-C/Apo B and HDL-C/Apo A_1 ratios). CRP levels were strongly and positively correlated with fibrinogen levels ($r = 0.47$; $P < 0.001$) and inversely correlated with albumin levels ($r = -0.32$; $P < 0.001$). Albumin and fibrinogen levels were moderately inversely correlated ($r = -0.24$; $P < 0.001$). CRP was also positively associated with body mass index and albumin was positively associated with blood pressure (data not shown).

Figure 2 shows the effect of adjustment for confounding factors on the associations between the different inflammatory markers and the risk of vascular mortality in all men and in men without prior disease. Adjustment for (i) classical vascular risk factors except lipids (smoking, drinking, BMI, blood pressure, and medication use); (ii) employment grade and (iii) lipids (cholesterol fractions, apolipoproteins and surrogate measures of particle size) had relatively little effect on the risk associations (attenuating them



Estimates are adjusted for age only. The vertical axes show hazard ratios on a proportional (i.e. logarithmic) scale. The 'prior disease' group includes men with a recall of MI, stroke, angina, diabetes or cancer. Figures are shown so that the same physical distance on each horizontal axis denotes the same proportional difference in each measurement. The area of each plotting symbol is proportional to the amount of statistical information (i.e. it is inversely proportional to the variance of the log hazard ratio), and the fitted lines through the points show the best inverse variance weighted fits (assuming a log-linear risk-relationship). The relative risk differences associated with 2 SD differences in usual levels of each measure are provided in Table 2.

Figure 1 Associations of vascular mortality vs. usual plasma measurements in all men (top panels) and separately according to prior disease (bottom panels).

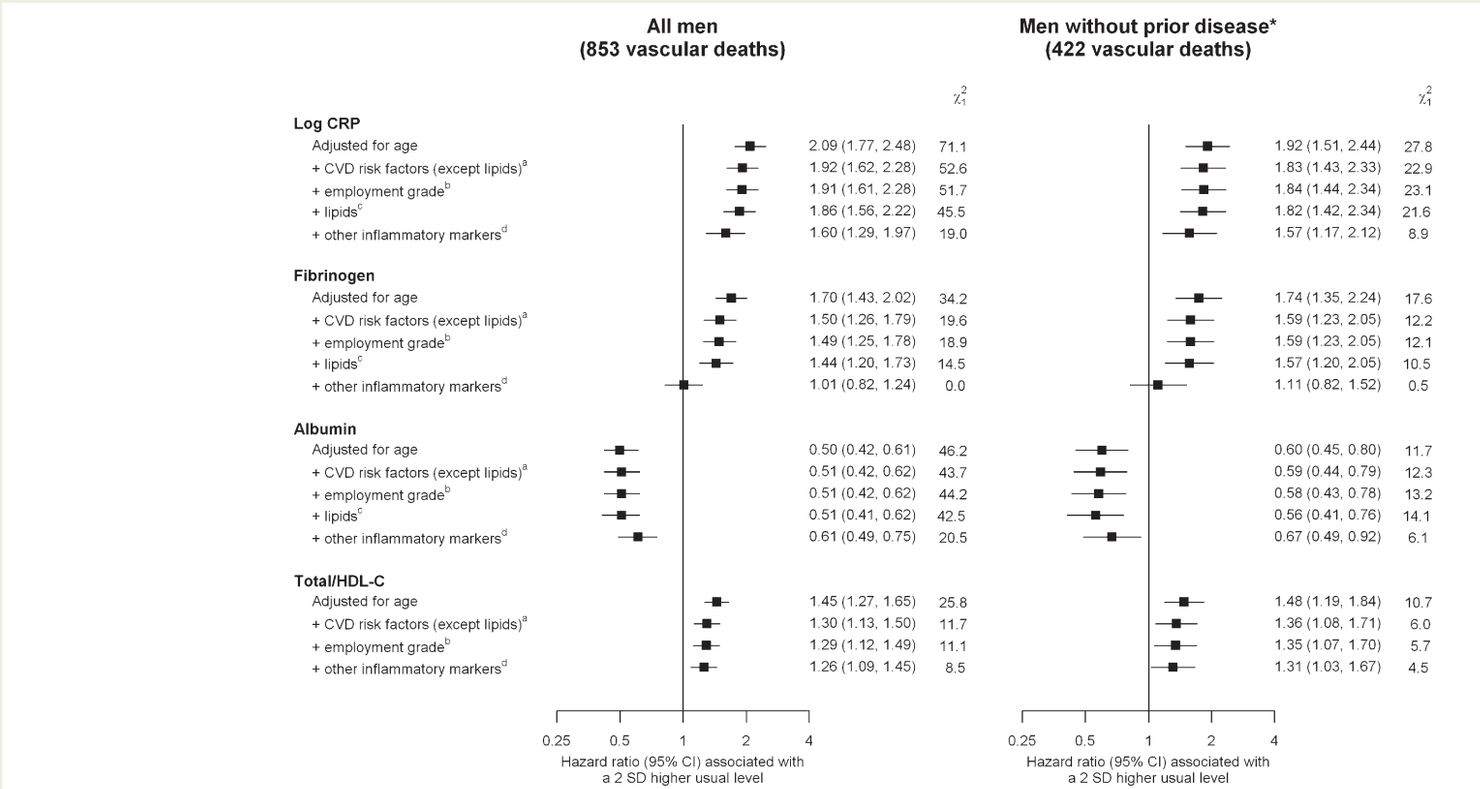
only slightly). The associations of vascular mortality with lnCRP (log C-reactive protein) were attenuated but remained significant after stepwise additional adjustment for other major risk factors excluding lipids (HR: 1.92; $\chi^2_1 = 53$), and when lipids and employment grade (HR: 1.86; $\chi^2_1 = 46$), and other inflammatory markers (HR: 1.60; $\chi^2_1 = 19$) were included. The substantial reduction in the χ^2_1 (from 71 to 19) for the associations of vascular mortality with lnCRP after making these adjustments suggests that confounding may have inflated the estimated strength of these associations. The reduction in the χ^2_1 statistic for the association of albumin with vascular mortality (from 46 to 21) after adjustment for other risk factors and inflammatory markers was comparable to that for lnCRP, but the associations of vascular mortality with fibrinogen became non-significant after additional adjustment for CRP and albumin (χ^2_1 reduced from 34 to 0). For non-vascular mortality, the effect of taking account of potential confounders was similar to the effects observed for vascular mortality (see Supplementary material online, *Figure S3*).

Utility of C-reactive protein measurements for prediction of overall survival

Figure 3 shows the estimated probability of survival from age 70 by history of prior disease and CRP level. The probability of surviving from age 70 to age 80 with or without elevated CRP (≥3 mg/L) was 77% vs. 89%, respectively, in men without prior disease; and 58% vs. 72% in men with prior disease. There was a difference of about 8 years in the median survival from age 70 in men with elevated CRP (≥3 mg/L) and prior disease (~82 years) compared with men with normal CRP levels and no prior disease (~90 years).

Discussion

In this study of 5360 older men, the positive association for a 2-SD difference in usual levels of lnCRP and the risk of vascular mortality



* no recall of a diagnosis of myocardial infarction, angina, stroke, diabetes or cancer; a: smoking status (current/ex/never); drinking status (current/non); body mass index; recall of a diagnosis of hypertension; mid blood pressure; use of aspirin, statins or blood pressure lowering medications; b: grade of employment at baseline; c: total cholesterol, HDL-C, apo A_1, apo B, LDL-C/Apo B ratio and HDL-C/$ApoA_1$ ratio; d: adjustment for the other two inflammatory markers presented.

Figure 2 Associations of vascular mortality vs. usual plasma levels of inflammatory markers before and after adjustment for confounding factors.

was stronger than that for an equivalent difference in usual levels of fibrinogen or total/HDL-C, and was comparable in magnitude to the strong inverse association observed for albumin (HR per 2-SD higher usual levels: 2.09, 1.70, 1.45, and 0.50, respectively). Adjustment for known vascular risk factors and the remaining biomarkers of inflammation attenuated the relative risk estimates for CRP (as shown by the reduction in χ^2_1 from 71 to 19) and albumin (from 46 to 21), but both remained significantly predictive of risk. For fibrinogen, however, the association with vascular mortality was non-significant after adjustment for CRP and albumin levels (χ^2_1 reduced from 34 to 0). CRP, albumin and, to a lesser extent, fibrinogen levels were also found to predict non-vascular mortality, with the magnitude of the risk associations being similar to those observed for vascular mortality. These non-vascular mortality associations were also preserved for CRP and albumin, but not for fibrinogen, after adjustment for vascular risk factors and other inflammatory factors (see Supplementary material online, *Figure S3*).

Consistency with previous studies

The strength of the association observed between CRP and vascular mortality in this study is generally consistent with previous studies of middle-aged populations.[10] In a recent meta-analysis of CRP and IHD risk in mainly middle-aged people (mean age 57 years), individuals in the top vs. the bottom tertile of CRP (an approximate 2-SD difference) were associated with a 58% higher risk of CHD.[10] However, few studies have examined the associations of CRP with vascular diseases in older people and the available evidence is conflicting, with some reporting positive associations[15,16] and others reporting no such associations.[17,18] The Cardiovascular Health Study[15,16] reported significant independent associations of CRP with non-fatal and fatal IHD events that were similar in magnitude to the present study, but did not provide comparisons with associations for blood lipids or directly assess associations with non-vascular mortality. The Rotterdam study reported no significant association of CRP with IHD risk but was based on only 157 IHD events and did not address associations with non-vascular mortality.[17]

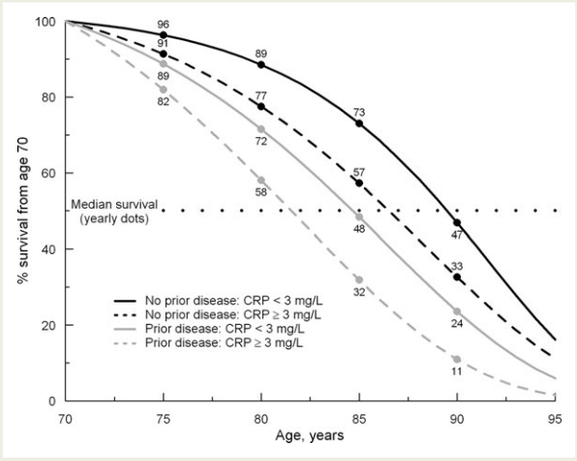


Figure 3 Estimated survival from age 70 in men, by history of prior disease and C-reactive protein level.

Overall, the present study involving 853 vascular and 1106 non-vascular events found that, after adjustment for baseline values of known risk factors other than lipids, lnCRP was more strongly associated with vascular mortality than was total/HDL-C [HR per 2-SD: 1.92 (95%CI 1.62–2.28) vs. 1.30 (1.13–1.50)], as was albumin (HR: 0.51) and fibrinogen (HR: 1.50). The associations of CRP with non-vascular mortality were unexpected and unexplained. Most previous studies of CRP and mortality have used a 'nested' case-control study design and were unable to provide comparisons of vascular and non-vascular mortality.[5–10,13] Considering vascular and non-vascular mortality together, a CRP level in excess of 3 mg/L was associated with about a 3-year difference in life expectancy at age 70 (both in men with and without known prior disease; *Figure 3*).

Fibrinogen has been shown in many previous studies to be positively related to both vascular and non-vascular mortality. A meta-analysis based on individual data from 31 prospective studies reported that fibrinogen was independently associated with vascular and non-vascular mortality, albeit the strength of these associations was attenuated by adjustment for established risk factors. The present study differs from this meta-analysis, not only by studying an older population but also by being able to control for a much wider range of established risk factors (as well as CRP and albumin). Taking these extra characteristics into account, in particular the additional inflammatory markers, removed the apparent association between fibrinogen and vascular (and non-vascular) mortality risk, by contrast with previous studies of middle-aged populations.[14]

Low albumin has been linked with susceptibility to vascular risk[28] and is used as a biochemical measure of frailty in older people.[29] A previous meta-analysis of 8 prospective studies of albumin and risk of IHD, involving 3770 IHD cases with a weighted mean age at baseline of 64 years, reported a HR of 1.5 (95%CI: 1.3–1.7) for IHD for individuals with albumin levels in the bottom compared with the top third.[28] Moreover, that meta-analysis reported associations of low albumin with all-cause mortality [OR: 1.9 (95%CI: 1.6-2.3)] and with total cancer mortality [OR: 1.9 (95%CI: 1.5–2.4)]. As with CRP and fibrinogen, in elderly individuals however, there have been relatively few studies linking low albumin to risk of vascular and non-vascular mortality.[28–30] The present study demonstrated that a low albumin level was a strong predictor for both vascular and non-vascular mortality, irrespective of prior disease, and that the strength of the associations were comparable with those for CRP.

Study limitations

The primary outcome under evaluation in the present study was vascular mortality rather than vascular events. While this outcome is less influenced by ascertainment bias due to variations in diagnostic practice, the attribution of cause of death is subject to misclassification of the cause of death. Misclassification of vascular deaths as non-vascular deaths might lead to underestimates of the magnitude of the relative risks associated with vascular mortality in relation to these biomarkers. However, the associations between CRP and vascular outcomes have previously been shown to be

similar for fatal and non-fatal outcomes in middle-aged individuals,[10] and the relative risks for vascular death and non-vascular death were similar in the present study.

Classification of prior disease was based on self-report, but as the relative risks associated with the different inflammatory markers were similar for vascular mortality among those with and without prior disease, the effect of any misclassification bias is again likely to be small. While it was possible to investigate the relations between the inflammatory markers and mortality risk after adjustment for classical risk factors (*Figure* 2), it is possible that baseline levels of these confounding risk factors do not accurately reflect usual levels (due to regression dilution bias) so that this adjustment did not take account of the full effects of these risk factors. In addition, no information was available on abdominal obesity or physical activity (factors known to affect both CRP concentrations and cardiovascular risk[3]) and, hence it was not possible to take them into account. It is, therefore, possible that residual associations of CRP with risk are due in part to these other characteristics (or, indeed, to some other unmeasured characteristics). Finally, minor differences between those who responded and those who did not respond to the re-survey were observed,[23] but it is unlikely that such differences would affect the validity of the present results.

Conclusions

This study demonstrates that both elevated plasma levels of CRP and low plasma levels of albumin are informative biomarkers of poor health status that predict both vascular and non-vascular mortality in older people, and retain their predictive value in the setting of prior disease. These associations may arise because CRP and albumin levels reflect underlying inflammatory disease or, perhaps, as a result of confounding due to their relationships with causal factors. Alternatively, it is possible that biomarkers of inflammation reflect a final common biochemical pathway of poor health status, (possibly triggered by cytokines) resulting in increased levels of CRP and decreased levels of albumin, which predispose to vascular and non-vascular mortality in old age.[30] This uncertainty should not, however, alter the utility of these markers for prediction of mortality. In particular, this study suggests that measurement of CRP and albumin in older people without established disease may provide a useful method of targeting older people for more intensive therapy to prevent vascular disease. Moreover, in view of the apparent importance of low-grade systematic inflammation for prediction of vascular and non-vascular mortality, additional randomized evidence is now required to assess effects on survival of low-dose aspirin or other vascular risk factor modification therapy in older people without prior vascular disease.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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