Relative value of multiple plasma biomarkers as risk factors for coronary artery disease and death in an angiography cohort

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∞ See related article page 479

Abstract

Background: Although elevated levels of C-reactive protein (CRP), interleukin (IL)-6, serum amyloid A protein (SAA) and total homocysteine (tHcy) have been associated with the increased likelihood of cardiovascular events, the relative or combined utility of these biomarkers in predicting atherosclerosis and death in an angiography cohort is unknown.

Methods: A cohort of 1117 consecutive patients (797 men and 320 women), referred to 2 Vancouver teaching hospitals for selective coronary angiography, was recruited between 1993 and 1995. Angiography results were obtained for 1019 patients. In 2004 we determined that of 1050 patients who could be traced, 231 had died, 95 of CAD-related causes. We compared the relative utility of baseline measurements of CRP, IL-6, SAA and tHcy as well as of lipids for predicting angiographic CAD and all-cause and CAD-related death.

Results: The risk of death increased across quartiles for CRP, IL-6, SAA and tHcy. When comparing the highest and lowest quartiles, the greatest hazard ratios were associated with IL-6 (2.57, 95% confidence interval [CI] 1.62–4.09) and tHcy (2.36, 95% CI 1.53–3.65). A Cox regression model containing all plasma biomarkers and traditional risk factors indicated that age, angiographic CAD and baseline plasma levels of IL-6 and tHcy remained independent predictors of CAD-related death, whereas age, sex, smoking, diabetes and apolipoprotein B levels were independent predictors of angiographic CAD. Kaplan–Meier survival curves indicated a utility in combining measures of CRP, SAA, IL-6 and tHcy for predicting risk of all-cause and CAD-related death.

Interpretation: A comparison of elevated levels of CRP, IL-6, SAA and tHcy with traditional CAD risk factors indicated that IL-6 and tHcy were the strongest independent biomarkers for CAD-related death. Elevated levels of multiple biomarkers were associated with an increasing rate of all-cause and CAD-related death.

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Several plasma biomarkers have been investigated to determine their utility as tools for predicting risk of cardiovascular disease. Elevated levels of C-reactive protein (CRP) have been shown to be a consistent predictor of cardiovascular events in both healthy and diseased populations.[1,2] However, the results of a recent report from the Reykjavik prospective study indicated that elevated CRP levels were only a moderate predictor of risk compared with established risk factors such as total cholesterol levels and cigarette smoking.[3] Although interleukin (IL)-6 is known to induce hepatic synthesis of CRP, there is evidence that elevated IL-6 and CRP plasma levels are independently related to several cardiovascular risk factors.[4] Like CRP and IL-6, serum amyloid A protein (SAA) is an acute-phase reactant that has been associated with coronary artery disease (CAD)[5–8] and is independent of other atherosclerotic risk factors for predicting 3-year risk of major cardiovascular events.[9] High plasma levels of total homocysteine (tHcy) have been associated with CAD-related death,[10,11] although the results of meta-analyses have indicated that tHcy is only a modest independent predictor of CAD.[12,13]

There is little data examining the comparative and combined utility of multiple plasma biomarkers for predicting long-term risk of CAD-related death. Since individual biomarkers have been shown to have independent biologic effects, it has been suggested that multiple measures could have additive value in cardiovascular risk assessment.[14] However, the relative utility of measuring multiple plasma biomarkers to predict risk of angiographic CAD and CAD-related death remains unclear. Therefore, we compared 4 common plasma biomarkers of CAD, specifically CRP, IL-6, SAA and tHcy, with traditional cardiovascular disease risk factors to assess their relative and cumulative value in predicting all-cause and CAD-related death.

Methods

A cohort of 1117 patients (797 men and 320 women) was recruited from a population of consecutive subjects referred for selective coronary angiography from 2 Vancouver teaching hospitals between 1993 and 1995. We have reported the genetic and biochemical analyses of this cohort previously.[15–17] Most (87%) of the patients in the cohort were white. All signed

an informed consent form; this research was approved by the Research Ethics Board of St. Paul's Hospital, Vancouver.

At the time of angiography, a 2-page questionnaire regarding clinical and lifestyle variables was administered to every patient by a nurse or attending cardiologist. Information was obtained on ethnicity, smoking status (ever, current and never) and alcohol consumption (never, 1–5, 6–10 and > 10 drinks per week). Weight, height, waist circumference and blood pressure were measured. A history of cardiovascular disease, diabetes, hypertension and renal insufficiency in the patient and the patient's family was obtained by self-report. Medications were recorded from the patients' charts.

Each angiogram was assessed semiquantitatively by a cardiologist blinded to any experimental results. Each lesion was assessed for percent diameter stenosis rounded to the nearest 10%. Patients with one or more lesions of more than 10% stenosis were considered to be positive for CAD, and patients with lesions of 10% or less stenosis were considered negative for CAD. This definition differs from that typically found in the literature, and it was chosen because there is increasing evidence that small plaques may contribute more than large plaques to cardiovascular morbidity and death.[18] In addition, by considering patients with any degree of narrowing to have CAD, we hoped to be able to avoid misclassifying patients as a result of subjective differences in diagnosis around the 50% mark. The original data collection did not permit the calculation of an angiography score.

Before the angiography procedure, fasting blood samples were collected in EDTA (ethylenediamine tetra-acetate) and centrifuged, and the plasma was divided into aliquots and stored at –70°C. Lipid and lipoprotein levels were measured soon after the subjects were recruited. Total cholesterol,[19] triglyceride,[20] high-density lipoprotein cholesterol[21] and apolipoprotein B[22] and A-I[22] levels were measured using previously described methods. Low-density lipoprotein cholesterol levels were calculated using the Friedewald formula[23] for patients whose plasma triglyceride level was less than 4 mmol/L.

In 2002 or later the frozen plasma samples were thawed and used to quantify the levels of CRP, SAA, IL-6 and tHcy. CRP was measured using a high-sensitivity CRP chemiluminescent enzyme-labelled immunometric assay using the IMMULITE 2000 automated analyzer (Diagnostic Products Corporation, Los Angeles, Calif.). The linear range of the assay is 0.2–150 mg/L, with a maximum interassay coefficient of variation (CV) of 8.7%. IL-6 was measured using the Quantikine High Sensitivity Human IL-6 Immunoassay (R&D Systems, Minneapolis, Minn.). The assay used the quantitative sandwich enzyme immunoassay technique with a linear range of 0.1–10 ng/L and a maximum interassay CV of 9.6%. SAA was measured using a high-sensitivity Human Serum Amyloid A ELISA kit (Antigenix America Inc., Huntingdon Station, NY). The linear range of the assay is 1–80 µg/L with a maximum interassay CV of 9%. tHcy was measured using the ADVIA Centaur HCY assay, which uses a competitive immunoassay and direct chemiluminescent technology. The linear range of the assay is 0.5–65 µmol/L with a maximum interassay CV of 7.6%. When necessary, plasma samples were diluted to obtain measurements in the linear range.

In July 2004, the names of the patients were linked with the British Columbia Vital Statistics database to determine

Table 1: Baseline characteristics of patients by outcome

Variable	Patient group; mean (SD)*		
	Alive *n = 819*	Deceased *n = 231*	*p* value
Age	59.3 (0.38)	66.0 (0.75)	< 0.001
TC, mmol/L	5.16 (0.04)	5.01 (0.08)	0.17
LDL-C, mmol/L	3.59 (0.03)	3.63 (0.07)	0.78
HDL-C, mmol/L	0.96 (0.01)	0.96 (0.02)	0.87
TC:HDL-C ratio	5.75 (0.08)	5.56 (0.12)	0.60
Triglycerides, mmol/L, median (IQR)	1.56 (1.13-2.20)	1.51 (1.10-1.99)	0.09
Apolipoprotein B, g/L	0.97 (0.01)	0.96 (0.02)	0.55
Waist circumference, cm	92 (1)	92 (1)	0.97
CRP, mg/L, median (IQR)	1.76 (0.81-4.73)	3.10 (1.28-6.79)	< 0.001
SAA, µg/L, median (IQR)	240 (96-672)	398 (163-1014)	< 0.001
IL-6, ng/L, median (IQR)	2.10 (1.30-3.44)	3.02 (2.00-4.90)	< 0.001
tHcy, µmol/L, median (IQR)	13.6 (10.8-16.7)	16.2 (12.6-20.9)	< 0.001
Categorical variables, no. (%)†			
Women	213 (75)	72 (25)	0.047
Men	606 (80)	148 (20)	
Has never smoked	244 (83)	51 (18)	0.044
Has smoked or does smoke	556 (77)	166 (23)	
No diabetes	694 (80)	173 (20)	0.031
Diabetes	125 (73)	47 (27)	
No hypertension	472 (80)	120 (20)	0.58
Hypertension	306 (78)	85 (22)	
Alcohol consumption			
Never	214 (78)	62 (22)	0.62
1-5 drinks/wk	430 (80)	106 (20)	
6-10 drinks/wk	134 (77)	40 (23)	
> 10 drinks/wk	22 (73)	8 (27)	

Note: SD = standard deviation, TC = total cholesterol, LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, IQR = interquartile range, CRP = C-reactive protein, SAA = serum amyloid A protein, tHcy = total homocysteine.
*Unless stated otherwise.
†Percentages are based on row totals.

Table 2: Hazard ratios* (HRs) for all-cause death according to quartiles of interleukin (IL)-6, serum amyloid A protein (SAA), C-reactive protein (CRP) and total homocysteine (tHcy) levels

Biomarker	Quartile				*p* value for trend†
	1	2	3	4	
IL-6					
Median, ng/L	0.99	1.81	2.85	5.63	
Quartile range, ng/L	≤ 1.37	1.37–2.27	2.27–3.77	≥ 3.77	
HR (95% CI)	1	1.60 (0.98–2.64)	1.40 (0.85–2.30)	2.57 (1.62–4.09)	0.001
SAA					
Median, μg/L	60	171	424	1773	
Quartile range, μg/L	≤ 104	104–271	271–743	≥ 743	
HR (95% CI)	1	1.40 (0.88–2.25)	2.01 (1.28–3.15)	2.01 (1.28–3.16)	0.001
CRP					
Median, mg/L	0.51	1.27	3.13	10.15	
Quartile range, mg/L	≤ 0.88	0.88–1.97	1.97–5.16	≥ 5.16	
HR (95% CI)	1	1.32 (0.83–2.09)	1.70 (1.08–2.66)	2.12 (1.38–3.27)	0.003
tHcy					
Median, μmol/L	9.6	12.6	15.4	21.4	
Quartile range, μmol/L	≤ 11.1	11.1–14.1	14.1–17.4	≥ 17.4	
HR (95% CI)	1	1.29 (0.80–2.08)	1.64 (1.04–2.61)	2.36 (1.53–3.65)	< 0.001

Note: CI = confidence interval.
*Adjusted for age, sex, total cholesterol:high-density lipoprotein cholesterol ratio, triglyceride and apolipoprotein B levels, waist circumference, diabetes, smoking status and hypertension.
†Quartiles were treated as a continuous variable to test if the regression coefficient is significantly different from zero.

whether they had died. Deaths occurring outside of the province of British Columbia were not identified or included in the analysis. CAD-related death was considered to have occurred when the underlying cause of death was attributed to ischemic heart disease according to the *International Classification of Disease, 10th revision* (ICD-10-CM codes I20–I25).[24]

Univariate analyses of continuous variables were carried out using the Mann–Whitney test for independent samples, and the χ^2 test was used to evaluate categorical variables. To assess the relation of IL-6, CRP, SAA and tHcy levels with death, Cox regression analyses were performed across quartiles where the first quartile was used as the reference category. To test for the presence of a trend across categories, quartiles were treated as a continuous variable in the Cox regression model. Comparison of area under the curve or receiver operating characteristic plots was performed using previously described methods.[25] To determine the strongest independent risk factors for CAD, multivariate logistic regression analyses were performed with age, sex, total cholesterol:high-density lipoprotein cholesterol (TC:HDL-C) ratio, triglyceride and apolipoprotein B levels, diabetes, waist circumference, hypertension, smoking status (ever or current), and CRP, SAA, IL-6 and tHcy levels included as covariates using a forward stepwise method. The end-point of survival analyses was all-cause death or death related to cardiovascular causes. The association of plasma biomarkers with outcome was evaluated by a Cox regression analysis adjusted for the same covariates mentioned above in addition to angiographic CAD using a forward stepwise method. The Kaplan–Meier test with log-rank statistics was used to evaluate differences in survival for increasing numbers of elevated plasma biomarkers.

Results

Of 1117 subjects recruited, angiography results were available for 1019. Data obtained from the British Columbia Vital Statistics database in July 2004 indicated that 231 patients (22%) had died, of whom 95 (9%) had died of CAD-related causes. The mean follow-up time was 8.5 years.

Many of the traditional risk factors for CAD, such as age, waist circumference, TC:HDL-C ratio and triglyceride and apolipoprotein B levels, were significantly higher in the CAD group than in the CAD-negative group, whereas HDL-C levels were significantly lower in the CAD group (Appendix 1, available at www.cmaj.ca/cgi/content/full/050880/DC1). Also, in categorical analyses, the proportions of men, patients who smoked and patients with diabetes were higher in the CAD group ($p < 0.001$ for all 3 variables) (Appendix 1). By contrast, CRP, SAA and IL-6 levels were not significantly different between the 2 groups, although higher levels were observed in the CAD group for each inflammatory marker. Comparison of tHcy levels indicated that significantly higher levels were observed in the CAD group ($p = 0.010$). If CAD was defined as having one or more lesions with greater than 50% stenosis and the reference group was patients with lesions of 50% or

less stenosis, statistical relations were similar to those reported in Appendix 1 with the exception that CRP and IL-6 levels were significantly higher in the CAD group ($p = 0.029$ and $p = 0.004$, respectively).

Comparison of alive and deceased groups indicated that baseline levels of CRP, IL-6, SAA and tHcy were elevated in the deceased group ($p < 0.001$ in all cases) (Table 1). No other plasma biomarker measured was associated with all-cause death. There was a higher proportion of women than of men in the deceased group (25% v. 20%, $p = 0.047$). Also, the frequency of smoking and diabetes was higher in the deceased group ($p = 0.044$ and 0.031, respectively) (Table 1). Patients who died of cardiovascular causes had higher levels of CRP, SAA, IL-6 and tHcy than those who were still alive ($p = 0.001$, $p = 0.003$, $p < 0.001$ and $p < 0.001$, respectively). No other plasma biomarkers or risk factors were significantly different between these 2 groups (data not shown).

After adjustment for age, sex, TC:HDL-C ratio, triglyceride and apolipoprotein B levels, waist circumference, diabetes, smoking status and hypertension, patients in the highest quartiles for IL-6, SAA, CRP and tHcy levels had a significantly increased risk of death (by a factor of 2.01–2.57) compared with those in the lowest quartile with significant trends across quartiles (Table 2). Further adjustments for use of lipid-lowering medication (17% frequency) and a self-report of renal insufficiency (3% frequency) did not substantially change this observation (data not shown).

Table 3: Odds ratios (ORs) for presence of coronary artery disease (CAD),* CAD-related death and all-cause death

Variable	Adjusted OR† (95% CI)	*p* value
Presence of CAD		
Age	1.05 (1.03–1.07)	< 0.001
Sex	5.40 (3.63–8.02)	< 0.001
Smoking status	2.03 (1.38–2.96)	0.003
Diabetes	2.74 (1.46–5.15)	0.002
Apolipoprotein B	1.36 (1.17–1.59)	0.001
CAD-related death		
Age	1.07 (1.05–1.10)	< 0.001
Angiographic CAD	3.52 (1.42–8.74)	0.007
IL-6	1.08 (1.01–1.16)	0.03
tHcy	1.05 (1.02–1.08)	0.002
All-cause death		
Age	1.05 (1.03–1.07)	< 0.001
Diabetes	1.53 (1.07–2.18)	0.02
IL-6	1.08 (1.04–1.13)	0.004
tHcy	1.05 (1.03–1.07)	< 0.001

Note: CI = confidence interval, IL-6 = interleukin-6, tHcy = total homocysteine.
*Presence of CAD was defined as ≥ 1 lesions of > 10% stenosis; patients with lesions of ≤ 10% stenosis were considered negative for CAD.
†Covariates evaluated included age, sex, total cholesterol:high-density lipoprotein cholesterol ratio, triglyceride levels, apolipoprotein B levels (0.2 g/L increment), waist circumference (5 cm increment), diabetes, hypertension, smoking (ever or current), and C-reactive protein, serum amyloid A protein, IL-6 and tHcy levels. Angiographic CAD was included as a covariate for analyses of predictor variables for all-cause and CAD-related death.

Appendices 2–4, available at www.cmaj.ca/cgi/content/full/050880/DC1, show the comparison of the area under the curve obtained for receiver operating characteristic curves plotted for all of the plasma biomarkers measured, including TC:HDL-C ratio, for their ability to predict death by any cause. Elevated levels of CRP, SAA, IL-6 and tHcy were better predictors of death by any cause than chance, whereas the receiver operating characteristic curves for lipid and apolipoprotein B levels were not significant. The area under the curve was greatest for IL-6 followed by tHcy, SAA, and CRP. The area under the curve for IL-6 was significantly greater than those observed for SAA and CRP. Receiver operating characteristic curve analysis for CAD-related death yielded similar results, where the largest area under the curve was observed for IL-6 and was significantly greater than that for CRP.

Age, male sex, diabetes and smoking status were independent predictors of CAD in a forward stepwise logistic regression model (Table 3). Apolipoprotein B was the only plasma biomarker that was an independent predictor of CAD, with an odds ratio of 1.36 (95% CI 1.17–1.59) for each increment of 0.2 g/L. A Cox regression model containing the same variables mentioned above as well as angiographic CAD indicated that age, angiographic CAD, and elevated IL-6 and tHcy levels were the strongest independent predictors of CAD-related death, whereas age, diabetes, and elevated IL-6 and tHcy levels were the strongest predictors of all-cause death. Hazard ratios of 1.08 (95% CI 1.01–1.16, $p = 0.03$) or an 8% increase in CAD-related death for each ng/L increase in IL-6 and 1.05 (95% CI 1.02–1.08, $p = 0.002$) or 5% increase in CAD-related death for each μmol/L increase in tHcy were observed for CAD-related death (Table 3).

Survival analysis was performed to determine whether elevated levels of multiple biomarkers in the fourth quartile were associated with decreased survival (Fig. 1). A significant trend was observed when comparisons were made between the presence of 0, 1, 2, 3 and 4 elevated levels of biomarkers for all-cause and CAD-related death ($p < 0.001$ in each case).

Interpretation

We analyzed the relative and combined utility of CRP, SAA, IL-6 and tHcy as predictors of angiographic CAD and death. In the study population, traditional CAD risk factors such as age, male sex, smoking status and diabetes were independent predictors of CAD. The only plasma biomarker indicative of CAD was apolipoprotein B. However, of all risk factors, angiographic CAD and elevated levels of IL-6 and tHcy emerged as the strongest independent predictors of CAD-related death. Survival analysis indicated the capacity to predict either all-cause or CAD-related death increased when elevated measures of CRP, SAA, IL-6 and tHcy were combined.

High apolipoprotein B levels and traditional CAD risk factors such as diabetes and smoking have recently been recognized as common among the global community.[26] In this

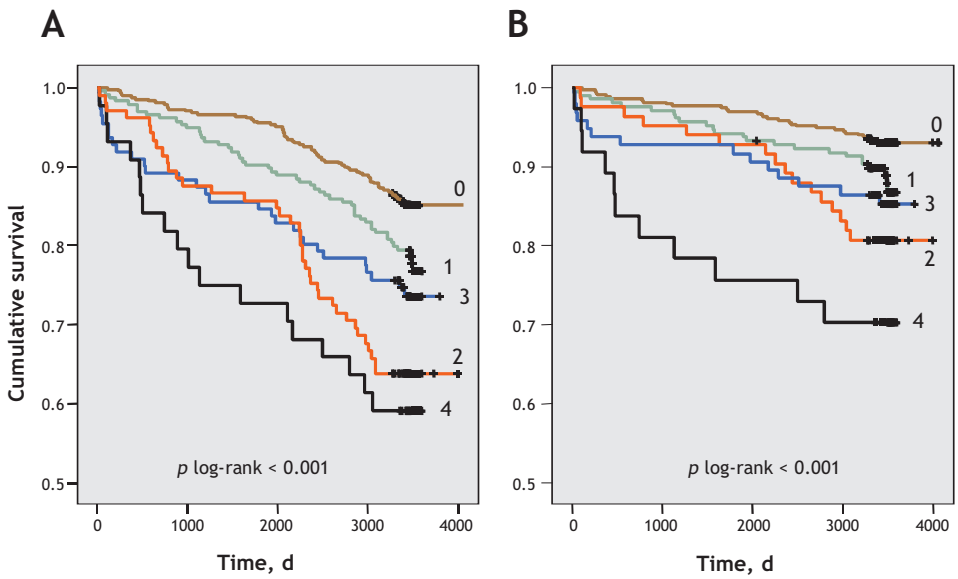

Fig. 1: Kaplan–Meier curves for all-cause and CAD-related death according to the number of plasma biomarkers (interleukin-6, serum amyloid A protein, C-reactive protein and total homocysteine) in the fourth quartile. A: The number of all-cause deaths per total number of patients in each category are as follows: 0 biomarkers, 69/471; 1, 51/235; 2, 38/105; 3, 29/111; 4, 18/44. B: The number of CAD-related deaths per total number of patients in each category are as follows: 0 biomarkers, 30/471; 1, 24/235; 2, 16/105; 3, 14/111; 4, 11/44.

study, none of CRP, SAA, IL-6 or tHcy was found to be an independent predictor of angiographic CAD. The observed relation between these 4 biomarkers and angiographic CAD is typically not strong, particularly in multivariate analyses, where either modest or no associations have been reported.[9,27–31] These data suggest that elevated apolipoprotein B levels are superior to those of CRP, SAA, IL-6 or tHcy in predicting the presence of atherosclerotic lesions.

These data indicate that IL-6 and tHcy have independent effects and are better predictors of CAD-related death than other inflammatory or lipid biomarkers. Consistent with this observation are previous reports of an elevated IL-6 level as an independent marker of increased risk of death and cardiovascular events in a variety of patient cohorts.[32–34] Furthermore, the results of previous studies have indicated that elevated IL-6 levels have stronger predictive value than elevated CRP levels for CAD-related death[35] and cardiovascular events.[36] Similarly, elevated tHcy levels have been shown to be a predictor of all-cause and CAD-related death,[10,11,37] and in 2 studies, this relation was independent of traditional risk factors and CRP.[38,39] However, elevated levels of CRP but not of tHCy or lipids were associated with sudden cardiac death in a 17-year follow-up of apparently healthy men.[40] The difference in results may be because the causes of sudden cardiac death are different from those of all-cause or CAD-related death.

Comparisons of survival between those subjects who had 0, 1, 2, 3 or 4 elevated markers among CRP, SAA, IL-6 and tHcy revealed an increasing rate of death and CAD-related death. As such, there appears to be increased prognostic value when measures of all 4 biomarkers are combined. A cumulative risk of 5 different inflammatory markers for coronary events was also recently reported in a cohort of 6075 apparently healthy men followed prospectively with a mean follow-up of 19 years.[41] However, in our study, it appears that these biomarkers may not be specific for CAD, since elevated IL-6 and tHcy levels were predictive of death by any cause.

There are several limitations to this study. First, it is important to note that the analyses described for this study cohort are based on the measurement of plasma biomarkers at a single point in time. We did not evaluate whether the levels of these markers changed with time or were influenced by medications after the angiography or by additional genetic and environmental factors. Angiography assesses only those lesions that protrude into the lumen and does not indicate CAD within outwardly modelled vessels. Furthermore, we did not measure other recognized indicators of inflammation such as elevated leukocyte counts and the erythrocyte sedimentation rate. Also, the lack of a measure of renal function prevented us from assessing the influence of this variable on biomarker levels and outcomes. Last, the relation of biomarker levels with outcomes was observed in patients referred for angiography and may not be directly applicable to the general population.

Although the results of this study indicate that the baseline measurement of IL-6 and tHcy as individual markers has greater utility in predicting death than traditional lipid and apolipoprotein measurements, IL-6 and tHcy do not appear to be specific predictors for CAD-related death. By contrast, greater prognostic value is achieved when IL-6 and tHcy are included as part of a group of biomarkers that together increase our ability to identify those subjects with greatest risk of death.

Editor's take

- Although, along with age and diabetes, anatomic severity of coronary artery disease is a strong predictor of subsequent death, a number of other plasma markers may be helpful in predicting death, particularly among patients who have undergone coronary angiography.

- In this study of over 1000 patients who had coronary angiograms and were followed for an average of 8.5 years, substantial increases in risk of death were associated with increasing levels of plasma C-reactive protein, interleukin (IL)-6, serum amyloid A protein and total homocysteine (tHcy).

Implications for practice: In the presence of known coronary artery disease, plasma biomarkers, in particular IL-6 and tHcy, are the strongest independent markers of subsequent CAD-related death.

This article has been peer reviewed.

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