

Adiponectin is an independent predictor of all-cause mortality, cardiac mortality, and myocardial infarction in patients presenting with chest pain

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IL-10

Aims To determine the prognostic value of baseline plasma adiponectin levels in patients with known or suspected coronary artery disease referred for coronary angiography.

Methods and results Adiponectin was measured in 325 male patients with stable angina, troponin-negative unstable angina, and non-ST-segment elevation myocardial infarction (MI) undergoing coronary angiography at a Veterans Administration Medical Center. The patients were then followed prospectively for the occurrence of all-cause mortality, cardiac mortality, and MI. Follow-up data at 24 months were available for 97% of the patients. Adiponectin was the only biomarker to independently predict the individual endpoints of all-cause mortality, cardiac mortality, and MI. The 24-month survival rates for patients in the lower (≤ 4.431 mg/L), middle (> 4.431 and ≤ 8.008 mg/L), and upper (> 8.008 mg/L) tertiles of plasma adiponectin values were 95.0, 90.4, and 83.5%, respectively ($P = 0.0232$ by log-rank test). Furthermore, when patients with chest pain were risk-stratified into those with and without a non-ST-segment elevation acute coronary syndrome (NSTEMI), adiponectin remained an independent predictor of both all-cause mortality and cardiac mortality in the NSTEMI subgroup.

Conclusion In a cohort of male patients undergoing coronary angiography, a single baseline determination of plasma adiponectin is independently predictive of the subsequent risk of death and MI.

Introduction

Obesity is pandemic in industrialized countries and has been implicated as a major cause of cardiovascular morbidity and mortality.^{1–4} Adipose tissue secretes a number of cytokines that may directly contribute to the development of obesity-related diseases, such as diabetes mellitus, dyslipidaemia, hypertension, and atherosclerotic vascular disease.^{5,6} Adiponectin is a recently discovered adipocyte-specific cytokine which, in contrast to other adipokines, has been described to have anti-inflammatory, anti-thrombotic, and anti-atherogenic properties.^{7–10} It is abundant in the plasma of normal subjects, but decreased in conditions such as obesity¹¹ and type-2 diabetes mellitus.¹² Furthermore, in healthy individuals, low plasma adiponectin levels have been associated with increased risk of cardiovascular events.¹³

Despite its apparent salutary effects, however, a recent prospective study in patients with chronic heart failure

found high, rather than low levels of plasma adiponectin to be an independent predictor of mortality.¹⁴ In addition, we and others have recently demonstrated that an elevated level of baseline plasma TIMP-1 is an independent predictor of mortality in patients with known or suspected coronary artery disease (CAD).^{15a,15b} This is relevant, as adiponectin has been shown to increase TIMP-1 levels through IL-10 expression in human macrophages.¹⁶ Taken together, the conflicting findings in diverse patient subgroups raise the possibility that adiponectin levels may have divergent prognostic implications in healthy vs. sick populations. To date, there have been no studies specifically examining the prognostic value of baseline plasma adiponectin levels in patients with known or suspected CAD presenting with chest pain. The purpose of the present study was to determine the prognostic significance of plasma adiponectin levels in a group of patients with stable angina, unstable angina, and non-ST-segment elevation myocardial infarction (NSTEMI) referred for coronary angiography in the context of other established inflammatory biomarkers such as high-sensitivity C-reactive protein (hs-CRP) and myeloperoxidase (MPO), as well as both IL-10 and TIMP-1.

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Methods

Study design

The present study represents an analysis of a subpopulation derived from a database comprised of 389 patients. The database was generated at an urban Veterans Administration (VA) Medical Center and was approved by the local IRB. All patients referred to the Cardiac Catheterization Laboratory for coronary angiography between January 13, 1999 and October 17, 2002 were eligible for inclusion in the database. Patients with active gastrointestinal bleeding or a haemoglobin concentration less than 8 g/dL were excluded. During the period of study enrolment, a total of 523 unique and consecutive male patients underwent diagnostic coronary angiography. Of these 523 patients, 50 could not be enrolled because of an unexpected loss of key study personnel between January 29, 2001 and July 2, 2001. Of the remaining 473 patients, 84 were either unwilling or unable to provide informed consent. Thus, a total of 389 patients provided informed consent and constituted the total population from which the database was derived. Clinical and demographic information was obtained on all patients by interview as well as review of the computerized medical records. Fasting blood was obtained from the patients at the time of angiography for subsequent analysis. The patients were then followed prospectively for the development of clinical events. For the present study, patients referred for coronary angiography for reasons other than the evaluation of chest pain (such as valvular heart disease, preoperative testing, and so on) were excluded from the analysis ($n = 41$), as were patients with recent (i.e. within 1 week) STEMI ($n = 23$). Thus, the final study population for the current report consisted of 325 patients with stable angina, troponin-negative unstable angina, and NSTEMI. The primary end-point of the study was all-cause mortality at 24 months for the entire cohort of 325 patients. The secondary endpoints of the study were cardiac mortality and myocardial infarction (MI) at 24 months for the entire cohort, as well as all-cause mortality, cardiac mortality, and MI (all at 24 months) for the stable angina and the non-ST-segment elevation acute coronary syndrome (NSTEMACS) subpopulations.

Blood sampling

After an overnight fast of at least 12 h, blood was obtained from all patients enrolled in the study. Blood was collected from the arterial sheath (after a 5 mL discard) at the time of angiography but prior to the injection of contrast material. Blood was immediately placed into vacutainer tubes, spun at 10 g for 20 min in a cold centrifuge and the plasma aliquoted into multiple 1.5 mL Eppendorf tubes. The samples were subsequently stored at -70°C until analysis at a later date.

Laboratory methods

Aliquoted plasma samples stored at -70°C were thawed, and using commercially available ELISA kits, the levels of the following biomarkers were measured: total TIMP-1 (EMD Biosciences, San Diego, CA, USA), hs-C-reactive protein (Life Diagnostics, West Chester, PA, USA), MPO (Assay Designs, Ann Arbor, MI, USA), adiponectin (R & D Systems, Minneapolis, MN, USA), IL-10 (Pierce Biotechnology, Rockford, IL, USA), and insulin (Linco Diagnostics, St Charles, MO, USA). The sensitivities of the TIMP-1, hs-C-reactive protein, MPO, adiponectin, IL-10, and insulin assays were 0.0096 ng/mL, 0.1 mg/L, 0.13 ng/mL, 0.246 ng/mL, 0.25 pg/mL, and 2 $\mu\text{U/mL}$, respectively. The intra-assay coefficients of variation for TIMP-1, hs-C-reactive protein, MPO, adiponectin, IL-10, and insulin were <5.2 , <7.6 , <5.5 , <4.7 , <4 , and $<7\%$, respectively.

Definition of risk factors and clinical syndromes

Diabetes was defined as clinically known and treated diabetes mellitus. Patients were diagnosed as hypertensive if they were

documented to have a blood pressure greater than 140/90 mmHg on two or more occasions or if they were already on anti-hypertensive therapy. Smoking was defined as the inhaled use of cigarettes, cigars, or pipes in any quantity. Smokers were classified as former only if they had not smoked at all in the 6 months preceding the date of angiography. BMI was calculated by dividing the weight of the patient in kilograms by the square of his height in meters. Congestive heart failure (CHF), on presentation, was defined as the presence of either radiographic or clinical evidence of pulmonary venous congestion within the preceding 24 h of angiography. MI on presentation was diagnosed by a history of chest discomfort and troponin I >1.0 ng/mL.

Angiographic data

All patients and their angiograms were graded as to the number of diseased coronary arteries, taking into account the left main (LM), left anterior descending (LAD), left circumflex (LCX), and right coronary arteries (RCA). A coronary artery was considered diseased if there was any obstructive lesion $\geq 50\%$ in that artery or one of its major (≥ 2.5 mm) branches. Stenosis severity was determined by visual estimation (in at least two orthogonal views) and all angiograms were read by two angiographers working independently. Any differences in interpretation were subsequently reconciled by a third reviewer. The operators reading the angiograms were blinded to the results of any subsequent laboratory analyses or to the development of clinical events at long-term follow-up. LV systolic function was assessed by contrast ventriculography and categorized as normal [ejection fraction (EF) $\geq 55\%$], mildly- (EF 45–54%), moderately- (EF 31–44%), or severely-reduced (EF $\leq 30\%$). These four categories of LV function were scored as 0, 1, 2, and 3, respectively.

Clinical endpoints

Patients were followed for the occurrence of death (all-cause and cardiac) and MI. MI during follow-up (i.e. as a clinical outcome) was defined by a history of chest pain with an associated elevation of either troponin I >1.0 ng/mL or troponin T >0.1 ng/mL. In the case of a fatal MI where enzymatic confirmation was not possible, the diagnosis was made on the basis of either a death certificate or a hospital record documenting MI as the cause of death. Follow-up was obtained via a combination of telephone contact, review of the computerized medical records, and subsequent clinic visits and hospitalizations. A death was classified as cardiac if the predominant and immediate cause was related to MI or ischaemia, arrhythmia, refractory CHF, or if the death was sudden and unexpected in nature. A death was categorized as non-cardiac if the major underlying pathophysiologic process leading to the demise was not related to the cardiovascular system, such as metastatic malignancy, sepsis, liver failure, or pulmonary embolism. The information regarding the aetiology and date of death was obtained using the following modalities: review of the death certificate, screening of the social security death index, conversation with the next of kin and/or primary physician, and most commonly, review of the VA computerized medical records. In each and every case, the cause and date of death were confirmed with the use of more than one modality.

Statistical methods

Patients were divided into tertiles according to their baseline adiponectin levels. Summary statistics for the continuous variables were presented both as means (with standard deviations) as well as medians (with interquartile ranges), and comparisons between the three groups were performed with the non-parametric Kruskal–Wallis test. Log-transformation was applied to all biomarkers in order to reduce the skewness and kurtosis of the data. Categorical data were summarized as frequencies and percentages, and comparisons between the three groups were performed with Pearson χ^2 test or Fisher's exact test.

Time-to-event at 24 months was presented with Kaplan–Meier curves for the individual endpoints of all-cause mortality, cardiac mortality, and MI. Comparisons between the three groups identified by tertiles of adiponectin were performed with the log-rank test.

The predictors of all-cause mortality, cardiac mortality, and MI at 24 months were identified by univariate Cox regression. The results were presented as hazard ratios and 95%CI. For each of the endpoints and for all of the patient groups analyzed, the following same baseline variables were studied by univariate analyses: age, family history of premature CAD, diabetes mellitus, hypertension, active tobacco use, history of tobacco use, LDL, HDL, triglycerides, serum creatinine, BMI, CHF on presentation, MI on presentation, ASA use, beta-blocker use, ACE-inhibitor use, statin use, angiotensin receptor blocker (ARB) use, prior CABG, number of diseased coronary arteries, LV function, HbA_{1c}, insulin, hs-C-reactive protein, MPO, TIMP-1, IL-10, and adiponectin. For the biomarkers, the hazard ratios represented an increase in 1 SD in the respective log-transformed biomarker. Adiponectin was analyzed both as a continuous variable and as a categorical variable, in the latter case comparing the two lower tertiles to the highest tertile. Only those univariate predictors with $P < 0.05$ were subsequently entered into multivariable models. Multivariable Cox proportional hazard analyses were then performed as stepwise regressions with backward elimination to identify the independent predictors. The selection of predictors for the various outcomes was also performed in stages, adjusting first for age, then for other clinical co-variables which were significant on univariate analysis, and finally for various biomarkers (including adiponectin) which were significant on univariate analysis. Adjustments for multiple testing were performed using the Hochberg-Bonferroni multiple-testing procedure.¹⁷

All analyses used two-sided tests with an overall significance level of $\alpha = 0.05$.

Results

Baseline characteristics

A total of 325 male patients were enrolled in the study. Two-year follow up data were available for 97% of the patients. The baseline clinical, laboratory, and angiographic characteristics of the study population stratified by the lower, middle, and upper tertiles of adiponectin values are shown in *Table 1*. The breakdown of the patient population based upon the primary indication for coronary angiography was as follows: NSTEMI = 84 patients (25.8%), troponin-negative unstable angina = 86 patients (26.5%), and stable angina pectoris = 155 patients (47.7%). Patients with either NSTEMI or troponin-negative unstable angina were collectively referred to as the NSTEACS group and comprised 52.3% of the total population. NSTEACS constitutes a well-recognized subgrouping of patients with chest pain in the cardiovascular literature.^{18–20}

Association of adiponectin with baseline clinical variables and other biomarkers

Elevated adiponectin levels were seen in association with older age, higher HDL levels, and CHF on presentation (*Table 1*). In addition, the levels of adiponectin were also positively correlated with those of TIMP-1. Specifically, the correlation between adiponectin and TIMP-1 was 0.25 ($P < 0.0001$ by Spearman rank correlation). In contrast, the presence of diabetes mellitus, the presence of hypertension, HbA_{1c} levels, insulin levels, triglyceride levels, and BMI were all inversely correlated with increasing adiponectin levels.

Clinical outcomes for the entire population

For the 97% of patients in whom 24-month follow-up data were available, there were a total of 33 deaths (10.3%), of which 20 (61%) were classified as cardiac in aetiology. Similarly, 46 (14.6%) had developed an MI (fatal or non-fatal) by 24 months.

Together with adiponectin and the other biomarkers, all baseline clinical, laboratory, and angiographic variables shown in *Table 1* that were significant for their association with clinical outcomes with $P < 0.05$ were entered in a backward stepwise multivariable Cox regression analysis. After adjustment for these factors, increasing adiponectin, analyzed as a continuous variable, was found to be a strong and independent predictor of long-term all-cause mortality (*Table 2*), with a hazard ratio of 1.76 (95%CI 1.21–2.56, $P = 0.0031$). Other independent predictors of all-cause mortality were the number of diseased coronary arteries and the serum creatinine (analyzed as a continuous variable). Importantly, adiponectin was an independent predictor of all-cause mortality even after adjustment for other biomarkers, including hs-C-reactive protein and TIMP-1, the latter of which was independently predictive of all-cause mortality if adiponectin was not included in the multivariable model. With respect to cardiac mortality, adiponectin was again an independent predictor of this outcome, with a hazard ratio of 2.10 (95%CI 1.29–3.42, $P = 0.0030$) (*Table 3*). Finally, adiponectin was the only biomarker which was independently predictive of MI, with a hazard ratio of 1.59 (95%CI 1.14–2.22, $P = 0.0064$) (*Table 4*).

Adiponectin was also analyzed as a categorical variable, comparing the upper tertile of adiponectin values to the lower two. Adjusting first for age, then for other clinically important covariates listed on *Table 1* that were significant on univariate analysis, and finally for the various biomarkers (including adiponectin) that were significant on univariate analysis, adiponectin was again a strong and independent predictor of each of the three endpoints. The hazard ratios with the 95%CI for the 24 month outcomes of all-cause mortality, cardiac mortality, and MI were 2.42 (1.21–4.86; $P = 0.0125$), 3.58 (1.40–9.16; $P = 0.0079$), and 2.05 (1.10–3.82; $P = 0.0232$), respectively.

Adjustments for multiplicity for each of the endpoints indicated that they all retained statistical significance using the Hochberg-Bonferroni procedure (whether adiponectin was analyzed as a continuous or as a categorical variable).

Kaplan–Meier survival analysis showed a significantly reduced survival in patients in the highest adiponectin tertile (*Figure 1*). At 24 months, the all-cause mortality rate was 5.0% in the lowest tertile, 9.6% in the middle tertile, and 16.5% in the highest tertile ($P = 0.0232$ by log-rank test). Similarly, at 24 months, the cardiac mortality rates were 2.0, 5.8, and 10.8% in the lower, middle, and upper tertiles, respectively ($P = 0.0270$ by log-rank test). Finally, the MI-free survival rates for the respective tertiles were 94.1, 84.2, and 80% ($P = 0.0120$ by log-rank test).

Clinical outcomes for the NSTEACS and the stable angina subpopulations

In an attempt to determine if the predictive power of adiponectin was either related to or affected by baseline risk, patients with chest pain were further stratified into those with and without an NSTEACS. The median hs-C-reactive

Table 1 Baseline characteristics according to tertiles of plasma adiponectin levels for the entire population

Characteristics	Statistics	Adiponectin ≤4.431 µg/mL (N = 104) ^a	Adiponectin >4.431 and ≤8.008 µg/mL (N = 104) ^a	Adiponectin >8.008 µg/mL (N = 104) ^a	P-value
Age	Mean (SD)	63.4 (9.4)	65.2 (9.1)	68.1 (9.9)	0.0002
	Median	64.6	66	69.1	
	(25th, 75th)	55.9, 70.8	57.1, 73.3	61.9, 74.7	
Race	Black (N) (%)	45 (43.3)	26 (25.0)	24 (23.1)	0.0119
	Hispanic (N) (%)	27 (26.0)	30 (28.9)	33 (31.7)	
	White (N) (%)	32 (30.7)	48 (46.2)	47 (45.2)	
Family history of premature CAD	(N) (%)	31 (29.8)	29 (27.9)	20 (19.2)	0.177
Diabetes mellitus	(N) (%)	54 (51.9)	50 (48.1)	37 (35.6)	0.0466
Hypertension	(N) (%)	93 (89.4)	81 (77.9)	91 (87.5)	0.0448
History of tobacco use	(N) (%)	87 (83.7)	83 (79.8)	85 (81.7)	0.7966
Active tobacco use	(N) (%)	34 (32.7)	28 (26.9)	25 (24.0)	0.385
LDL	Mean (SD)	106.6 (32.0)	107 (37.5)	107.6 (33.5)	0.9013
	Median	105	104.3	107	
	(25th, 75th)	(82.6, 129)	(84.4, 124.6)	(86.4, 126.6)	
HDL	Mean (SD)	36.4 (7.8)	40.4 (13.8)	40.3 (10.5)	0.0153
	Median	35	40	40	
	(25th, 75th)	(31, 42)	(31, 46)	(33, 47)	
Triglycerides	Mean (SD)	174.8 (108.8)	141.4 (109.7)	123.7 (64.5)	0.0004
	Median	138	115.5	110.5	
	(25th, 75th)	(100.0, 214.5)	(71.5, 161.0)	(76.0, 162.5)	
Serum creatinine	Mean (SD)	1.10 (0.27)	1.26 (1.45)	1.34 (1.29)	0.7524
	Median	1.05	1.1	1.1	
	(25th, 75th)	(0.9, 1.2)	(0.9, 1.2)	(0.9, 1.2)	
BMI	Mean (SD)	30.1 (5.8)	28.4 (5.4)	28.4 (5.6)	0.0308
	Median	29.7	27.4	27.8	
	(25th, 75th)	(25.9, 33.9)	(25.2, 31.3)	(24.5, 31.2)	
CHF on presentation	(N) (%)	9 (8.7)	18 (17.3)	34 (32.7)	<0.0001
MI on presentation	(N) (%)	26 (25)	28 (26.9)	28 (26.9)	0.936
ASA use	(N) (%)	94 (90.4)	89 (85.6)	87 (83.7)	0.342
Beta-blocker use	(N) (%)	78 (75.0)	70 (67.3)	69 (66.4)	0.3313
ACE-inhibitor use	(N) (%)	64 (61.5)	60 (57.7)	54 (51.9)	0.37
Statin use	(N) (%)	65 (62.5)	51 (49.0)	57 (54.8)	0.1466
Fibrate use	(N) (%)	9 (8.7)	3 (2.9)	3 (2.9)	0.0823
ARB use	(N) (%)	7 (6.7)	10 (9.6)	3 (2.9)	0.1385
Prior CABG	(N) (%)	8 (7.7)	11 (10.6)	12 (11.5)	0.6277
Number of diseased coronary arteries ^b	0	23 (22.1)	15 (14.4)	19 (18.3)	0.3551
	1	19 (18.3)	14 (13.5)	18 (17.3)	
	2	29 (27.9)	25 (24.0)	27 (26.0)	
	3	31 (29.8)	41 (39.4)	35 (33.7)	
	4	2 (1.9)	9 (8.7)	5 (4.8)	
LV function	0 (EF ≥ 55%)	44 (45.4)	34 (35.8)	41 (41.4)	0.2412
	1 (EF 45–54%)	24 (24.7)	29 (30.5)	22 (22.2)	
	2 (EF 31–44%)	23 (23.7)	19 (20.0)	19 (19.2)	
	3 (EF ≤ 30%)	6 (6.2)	13 (13.7)	17 (17.2)	
Troponin I	Mean (SD)	4.0 (12.3)	6.2 (19.8)	6.1 (24.0)	0.6067
	Median	0.3	0.3	0.3	
	(25th, 75th)	(0.5, 1.8)	(0.2, 1.7)	(0.2, 0.8)	
hs-C-reactive protein	Mean (SD)	14.5 (19.6)	24.1 (40.4)	17.8 (32.4)	0.6764
	Median	9.5	8.5	8.6	
	(25th, 75th)	(3.9, 17.0)	(3.5, 20.2)	(2.8, 15)	
MPO	Mean (SD)	23.5 (22.4)	24.2 (15.9)	27.2 (20.5)	0.1723
	Median	17.2	19.8	19.2	
	(25th, 75th)	(12.6, 26.3)	(13.6, 28.7)	(13.9, 30.7)	
TIMP-1	Mean (SD)	78.7 (22.5)	87.7 (36.2)	100.2 (50.4)	0.0009
	Median	73.4	79.7	86.4	
	(25th, 75th)	(63.1, 93.8)	(66.1, 93.3)	(72.1, 106.7)	
IL-10	Mean (SD)	13.5 (26.2)	16.2 (28.3)	20.0 (40.5)	0.4702
	Median	5.7	6.4	5.9	
	(25th, 75th)	(4.4, 9.3)	(4.5, 11.6)	(4.7, 11.8)	
Insulin	Mean (SD)	12.8 (7.5)	11.8 (9.5)	11.3 (16.2)	<0.0001
	Median	11.8	7.8	7.1	

Continued

Table 1 *Continued*

Characteristics	Statistics	Adiponectin ≤4.431 µg/mL (N = 104) ^a	Adiponectin >4.431 and ≤8.008 µg/mL (N = 104) ^a	Adiponectin >8.008 µg/mL (N = 104) ^a	P-value
HbA _{1c}	(25th, 75th)	(7.2, 15.6)	(6.0, 13.5)	(5.4, 10.1)	0.0374
	Mean (SD)	7.0 (1.9)	6.6 (1.7)	6.4 (1.7)	
	Median	6.9	6	5.7	
	(25th, 75th)	5.6, 7.7	5.3, 7.3	5.3, 7.3	

^aBecause of 13 missing values for adiponectin, N = 104 patients for each tertile.

^bTakes into account the LM, LAD, LCX, and RCA.

Table 2 Multivariable Cox proportional hazard analysis for all-cause mortality for the entire population

Variable	P-value	Hazard ratio and 95%CI
Serum creatinine	0.0248	1.19 (1.02, 1.38)
Number of diseased coronary arteries	0.0012	1.88 (1.28, 2.76)
Adiponectin	0.0031	1.76 (1.21, 2.56)

Adiponectin and serum creatinine were modelled as continuous variables. Hazard ratio per 1 SD increment of log-transformed marker: adiponectin, 1 SD = 0.65 mg/L. For serum creatinine (not log-transformed), 1 SD = 1.1 mg/L. Multivariable Cox proportional hazard analyses were performed as stepwise regressions with backward elimination. Twenty-eight clinical, laboratory, and angiographic variables were initially studied (see *Table 1* and *Methods*) by univariate analysis. Only those predictors with $P < 0.05$ (age, serum creatinine, number of diseased coronary arteries, TIMP-1, hs-C-reactive protein, and adiponectin) were subsequently entered into a multivariable model, the results of which are displayed above.

Table 3 Multivariable Cox proportional hazard analysis for cardiac mortality for the entire population

Variable	P-value	Hazard ratio and 95%CI
Serum creatinine	0.0510	1.23 (1.00, 1.51)
Number of diseased coronary arteries	0.0073	2.01 (1.21, 3.35)
Adiponectin	0.0030	2.10 (1.29, 3.42)

Adiponectin and serum creatinine were modelled as continuous variables. Hazard ratio per 1 SD increment of log-transformed marker: adiponectin, 1 SD = 0.65 mg/L. For serum creatinine (not log-transformed), 1 SD = 1.1 mg/L. Multivariable Cox proportional hazard analyses were performed as stepwise regressions with backward elimination. Twenty-eight clinical, laboratory, and angiographic variables were initially studied (see *Table 1* and *Methods*) by univariate analysis. Only those predictors with $P < 0.05$ (age, serum creatinine, number of diseased coronary arteries, and adiponectin) were subsequently entered into a multivariable model, the results of which are displayed above.

protein values for these two groups of patients were 12.1 and 6.8, respectively ($P < 0.0001$). The NSTEACS group was comprised of those with unstable angina and NSTEMI. For this group, in whom follow-up data at 24 months were available for 100% of the patients, there were a total of 23 deaths (13.7%), of which 12 (52%) were classified as cardiac in aetiology.

As was done for the entire cohort of patients, adiponectin and all baseline clinical, laboratory, and angiographic variables shown in *Table 1* (including other biomarkers) were

Table 4 Multivariable Cox proportional hazard analysis for MI for the entire population

Variable	P-value	Hazard ratio and 95%CI
Age/10 years	0.0493	1.47 (1.00, 2.15)
MI on presentation	0.0416	1.92 (1.03, 3.59)
Number of diseased coronary arteries	0.0258	1.42 (1.04, 1.94)
Adiponectin	0.0064	1.59 (1.14, 2.22)
Beta-blocker use at baseline	0.0407	2.53 (1.04, 6.14)

Adiponectin was modelled as a continuous variable. Hazard ratio per 1 SD increment of log-transformed marker: adiponectin, 1 SD = 0.65 mg/L. Multivariable Cox proportional hazard analyses were performed as stepwise regressions with backward elimination. Twenty-eight clinical, laboratory, and angiographic variables were initially studied (see *Table 1* and *Methods*) by univariate analysis. Only those predictors with $P < 0.05$ (age, serum creatinine, MI on presentation, diabetes mellitus, beta-blocker use at baseline, number of diseased coronary arteries, TIMP-1, and adiponectin) were subsequently entered into a multivariable model, the results of which are displayed above.

entered in a backward stepwise multivariable Cox regression analysis. After adjustment for these factors, increasing adiponectin, analyzed as a continuous variable, was found to be a strong and independent predictor of long-term all-cause mortality (*Table 5*), with a hazard ratio of 1.778 (95%CI 1.133–2.788, $P = 0.0122$). The only other independent predictor of all-cause mortality in this subpopulation was baseline plasma hs-C-reactive protein levels. Similarly, with respect to cardiac mortality, increasing adiponectin (analyzed as a continuous variable) was found to be an even stronger independent predictor of this outcome (*Table 5*), with a hazard ratio of 2.258 (95%CI 1.223–4.171, $P = 0.0092$).

As was done for the entire cohort of patients, adiponectin was also analyzed as a categorical variable, comparing the upper tertile of adiponectin values to the lower two. Adjusting again in stages first for age, then for statistically significant clinical covariates listed in *Table 1*, and finally for the various biomarkers that were significant on univariate analysis, adiponectin remained a strong and independent predictor of both all-cause and cardiac mortality in the NSTEACS subpopulation. The hazard ratios with the 95%CI for the 24-month outcomes of all-cause mortality and cardiac mortality were 2.46 (1.06–5.69; $P = 0.0357$) and 3.67 (1.07–12.53; $P = 0.0382$), respectively.

Adjustments for multiplicity for each of the endpoints indicated that they both retained statistical significance using the Hochberg-Bonferroni procedure (whether adiponectin was analyzed as a continuous or as a categorical variable).

Similar to the total population of patients, Kaplan–Meier survival analysis for the NSTEMI subpopulation showed a significantly reduced survival in patients in the highest adiponectin tertile (Figure 2). At 24 months, the all-cause mortality rates were 7.7% in the lowest tertile, 11.3% in the middle tertile, and 23.1% in the highest tertile ($P=0.0613$ by log-rank test). Similarly, at 24 months, the cardiac mortality rates were 1.9, 6.7, and 13.7% in the lower, middle, and upper tertiles, respectively ($P=0.0487$ by log-rank test).

In the lower risk stable angina subgroup, adiponectin was not a predictor of all-cause mortality, cardiac mortality, or MI.

Discussion

In the present study, we demonstrate for the first time that adiponectin is a powerful and independent predictor of each of the individual endpoints of all-cause mortality, cardiac mortality, and MI in a high-risk cohort of 325 male patients consisting of stable angina, unstable angina, and NSTEMI referred for cardiac catheterization. Furthermore, in the higher-risk NSTEMI subpopulation, adiponectin remains an independent predictor of each of the individual endpoints of cardiac mortality and all-cause mortality.

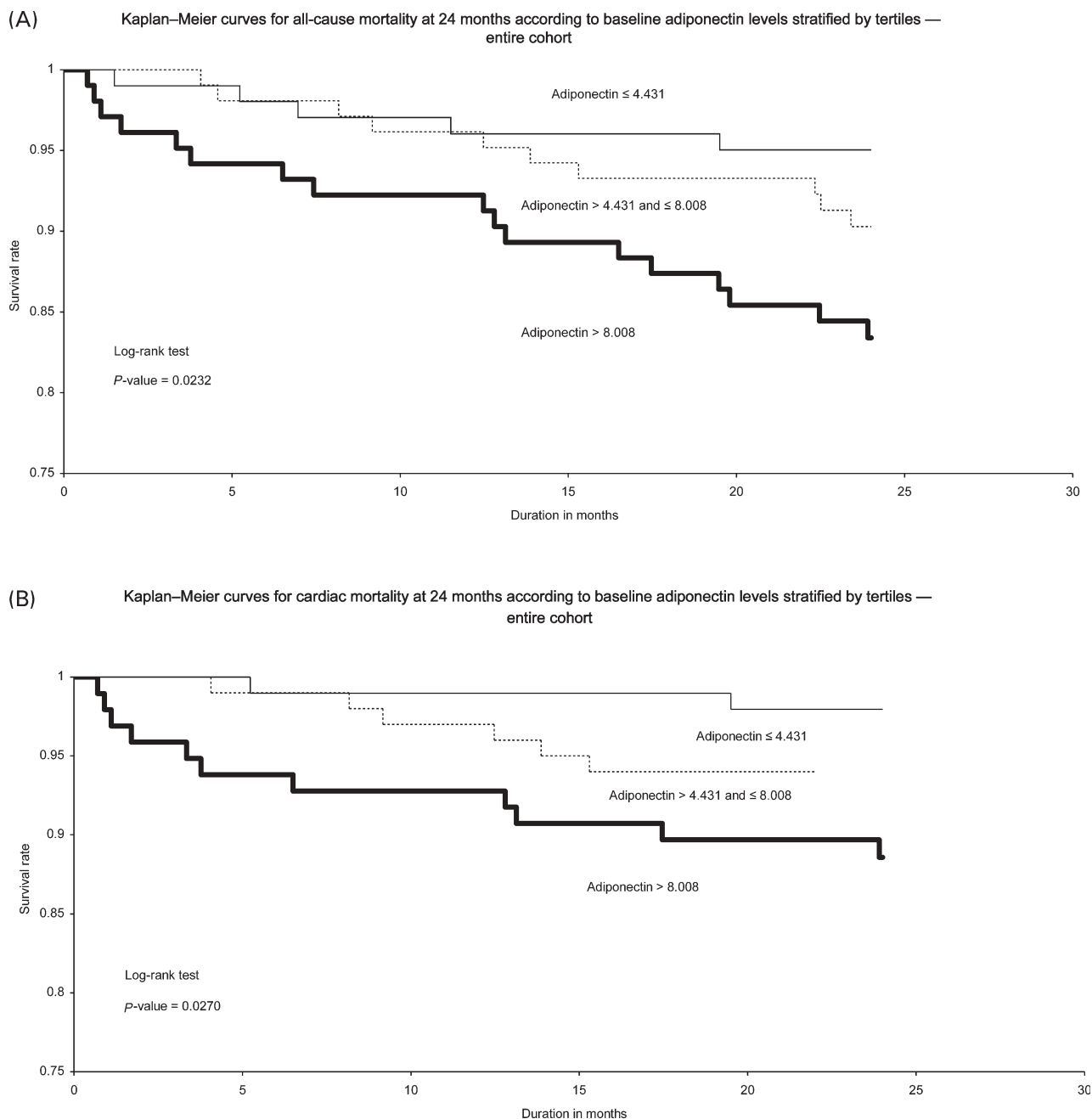


Figure 1 Kaplan–Meier curves for (A) all-cause mortality, (B) cardiac mortality, and (C) MI-free survival (time-to-first MI) according to baseline plasma adiponectin levels stratified by tertiles in the entire cohort of patients. At 24 months, the number of events for the lower, middle, and upper tertiles for (A) all-cause mortality were 5 (5.0%), 10 (9.6%), and 17 (16.5%), respectively. The number of events for the lower, middle, and upper tertiles for (B) cardiac mortality were 2 (2.0%), 6 (5.8%), and 11 (10.8%), respectively. The number of events for the lower, middle, and upper tertiles for (C) MI-free survival (time-to-first MI) were 6 (5.9%), 16 (15.8%), and 20 (20.0%), respectively.

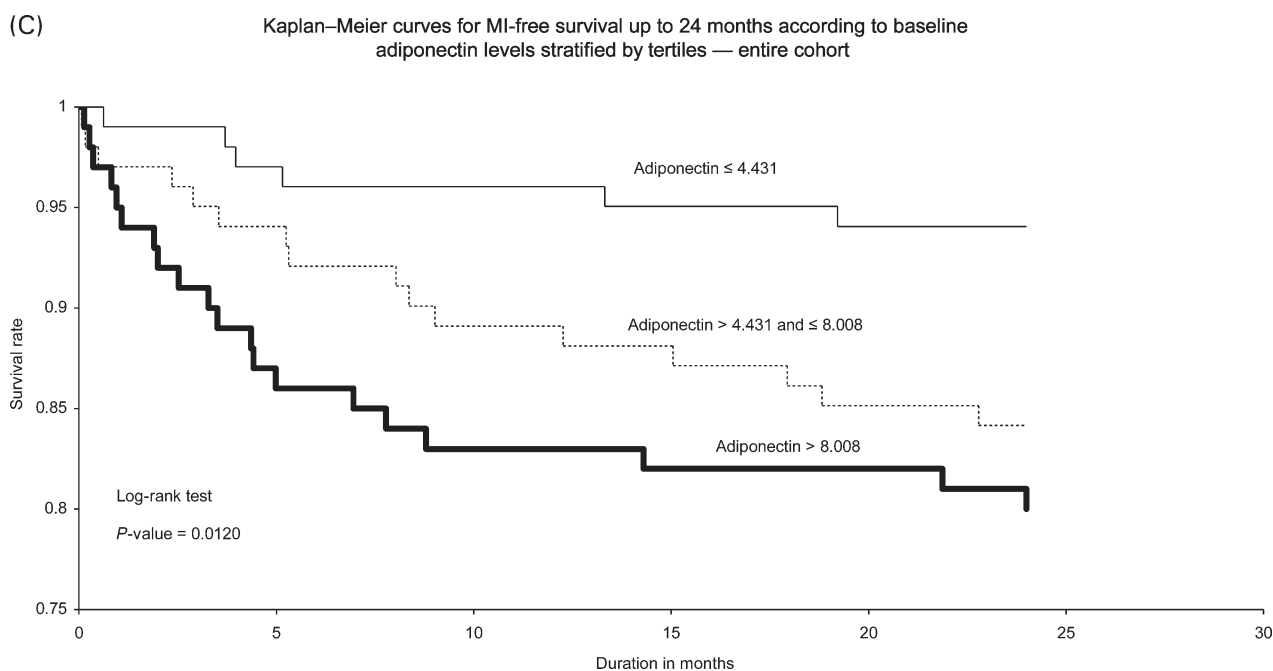


Figure 1 Continued.

Table 5 Multivariable Cox proportional hazard analyses for all-cause mortality and cardiac mortality for the NSTEACS subpopulation

Variable	P-value	Hazard ratio and 95%CI
All-cause mortality		
hs-C-reactive protein	0.0046	1.979 (1.234, 3.174)
Adiponectin	0.0122	1.778 (1.133, 2.788)
Cardiac mortality		
Adiponectin	0.0092	2.258 (1.223, 4.171)

Adiponectin and hs-C-reactive protein were modelled as continuous variables. Hazard ratios per 1 SD increment of log-transformed marker: adiponectin, 1 SD = 0.64 mg/L; hs-C-reactive protein, 1 SD = 1.37 mg/L. Multivariable Cox proportional hazard analyses were performed as stepwise regressions with backward elimination. Twenty-eight clinical, laboratory, and angiographic variables were initially studied (see *Table 1* and *Methods*) by univariate analysis. Only those predictors with $P < 0.05$ (age, number of diseased coronary arteries, TIMP-1, hs-C-reactive protein, and adiponectin for all-cause mortality; age and adiponectin for cardiac mortality) were subsequently entered into multivariate models, the results of which are displayed above.

Importantly, in this study we confirm many of the metabolic associations reported previously with adiponectin.^{11,12,21–23} Specifically, in our study, lower levels of adiponectin were seen in association with increasing BMI, increasing insulin levels, increasing HbA_{1c} levels, hypertriglyceridaemia, the presence of hypertension, and the presence of diabetes mellitus. In this regard, our findings are in accord with the literature and support the validity of our dataset. We also report a positive correlation between adiponectin levels and CHF, which is also in accord with the recent observations by Kistorp *et al.*¹⁴ Most importantly, our findings extend the observations of Kistorp *et al.* about the prognostic power of high adiponectin levels in CHF patients with respect to cardiovascular mortality to a population of patients with chest pain, including those with NSTEACS.

Our findings with respect to the positive correlation between adiponectin levels and adverse outcomes are in contrast to those of Pischon *et al.*¹³ who found low levels of adiponectin to be an independent predictor of adverse cardiovascular events in a population of healthy male patients. These conflicting observations may relate, at least in part, to the markedly different risk profiles of the populations in our study and that by Pischon *et al.* In the study by Pischon *et al.*, all patients were free of diagnosed cardiovascular disease at the time of blood draw. The lower risk profile of that particular population was also evident by the lower event rate, limited number of participants with diabetes at baseline, and the low baseline levels of C-reactive protein. In contrast, our study population consisted of a relatively high-risk cohort as manifest not only in the baseline clinical, angiographic, and laboratory data, but also in the high incidence of death and MI at 24 months. Furthermore, the median hs-C-reactive protein value for our cohort (8.7 mg/L) approximates that of a previously reported level (10 mg/L) used to define a population at high risk.²⁴ Although the inflammatory profile of the heart failure patients in the study by Kistorp *et al.* was not specifically reported, it is well-established that patients with CHF, like their ACS counterparts, are in a heightened inflammatory state.^{25–27} Therefore, it is conceivable that, under normal and non-inflammatory conditions, higher baseline levels of adiponectin may in fact be beneficial and both a marker and mediator of decreased cardiovascular risk. However, in a high-risk population with either active vascular or myocardial remodelling (such as that which occurs in the ACS or CHF), there may be a counter-regulatory or a compensatory increase in adiponectin levels. Alternatively, the increased levels may be a consequence of resistance at the level of the adiponectin receptor, a mechanism potentially akin to that seen in diabetics with elevated insulin levels. Thus, in such conditions elevated adiponectin levels may be a marker of either the underlying

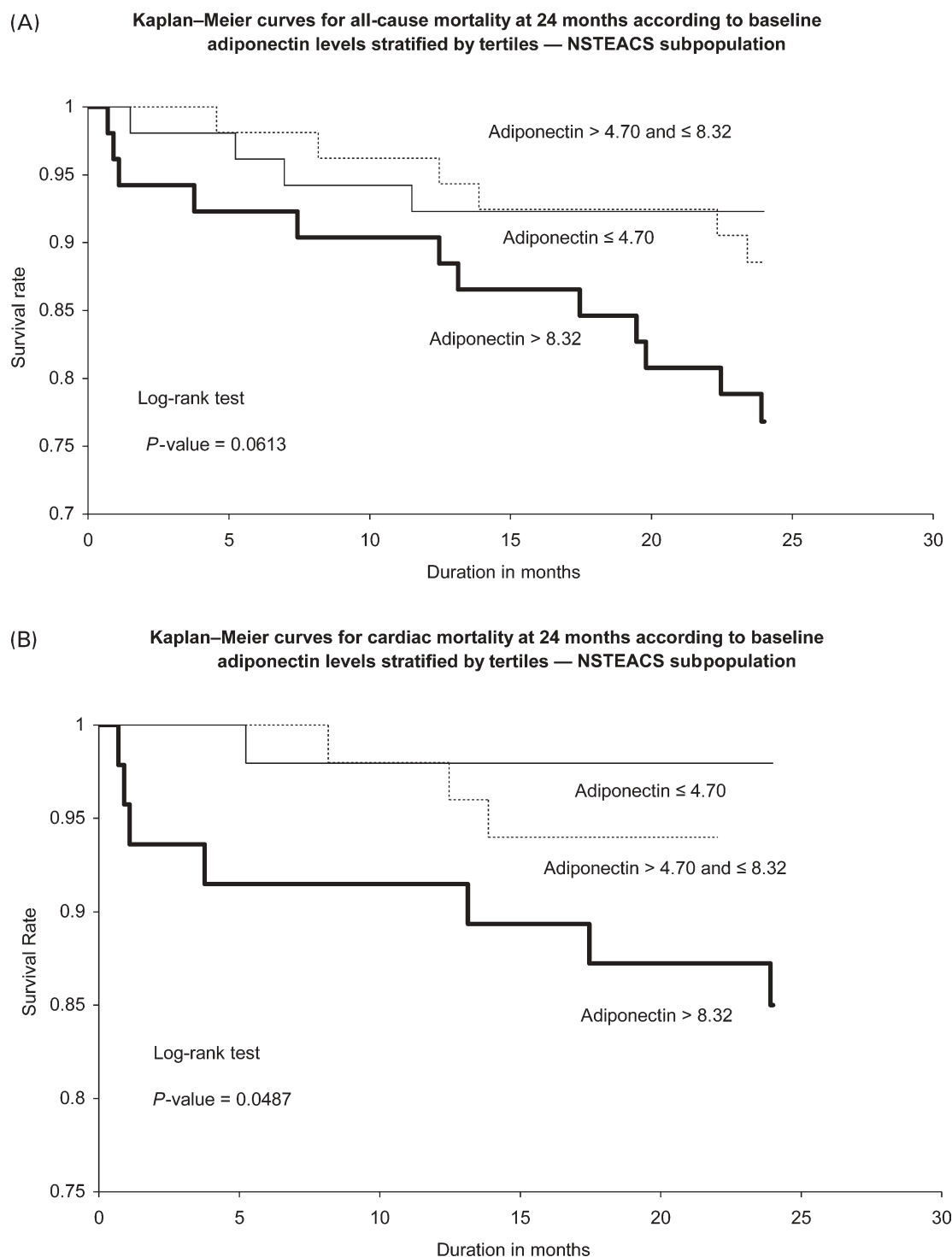


Figure 2 Kaplan–Meier curves for (A) all-cause mortality and (B) cardiac mortality according to baseline plasma adiponectin levels stratified by tertiles in the subpopulation of patients with NSTEACS. At 24 months, the number of events for the lower, middle, and upper tertiles for (A) all-cause mortality were 4 (7.7%), 6 (11.3%), and 12 (23.1%), respectively. The number of events for the lower, middle, and upper tertiles for (B) cardiac mortality were 1 (1.9%), 3 (6.7%), and 7 (13.7%), respectively.

inflammatory state or of adiponectin resistance. In this regard, the discrepancy between the *in vitro* and clinical observations with respect to adiponectin may be similar to that seen with TIMP-1. For example, experiments in animal models have demonstrated beneficial effects of TIMP-1 in terms of reducing intimal hyperplasia,²⁸ atherosclerotic lesions,²⁹ and aneurysm development.³⁰ However,

despite these beneficial effects, it has very recently been shown that high- and not low levels of TIMP-1 are associated with adverse cardiovascular outcomes in patients with known or suspected CAD.^{15a,15b} The case of TIMP-1 is particularly relevant, not only because TIMP-1 and adiponectin both have imputed anti-atherogenic properties, but also because these two proteins share the same pathophysiologic

pathway. Specifically, adiponectin has been shown to selectively increase the expression of TIMP-1 at both the mRNA and protein levels in human monocyte-derived macrophages.¹⁶ Indeed, in the present study, we demonstrate a statistically significant (albeit weak) positive correlation between the plasma values of these two biomarkers. Thus, it is not surprising that the levels of these two proteins were concordant in both their directionality and ability to predict adverse cardiovascular outcomes.

Study limitations

This study has a number of limitations. First, it was conducted in an exclusively male population. Secondly, the population was a high-risk cohort as evidenced by both clinical and laboratory parameters as well as by the high event rate for death and MI. Therefore, it is unknown whether adiponectin levels (either high or low) would be similarly predictive of events in a low-risk chest pain population. Although we were unable to demonstrate any prognostic significance of adiponectin in our stable angina subset, this may have related to issues of sample size or low event rates in this particular patient subgroup. Thirdly, it is unknown whether long-term storage of blood samples affects plasma adiponectin levels, and whether multiple measurements of adiponectin, as opposed to a single assessment, would provide more reliable results. Fourthly, the assay used in our study measured total plasma adiponectin levels and we were therefore unable to determine the levels of the various isoforms of adiponectin. Finally, the size of our population was small and the study was not designed with *a priori* calculations with respect to sample size or statistical power. As such, the findings need to be confirmed in larger and prospectively designed studies.

In conclusion, we found that high baseline plasma adiponectin levels are independently associated with an increased risk of both death and MI (as individual endpoints) at 2-year follow-up in a cohort of men with stable angina, unstable angina, and NSTEMI referred for coronary angiography. Furthermore, the prognostic ability of adiponectin in this regard is independent of inflammatory markers, such as hs-C-reactive protein. Importantly, this study is consistent with the recent observations that high, rather than low, adiponectin levels may predict adverse outcomes in high-risk populations in whom there is an underlying heightened inflammatory state.

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