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Authors: Krystian Mróz, Elżbieta Paszek, Mateusz Baran, Michał Ząbczyk, Saulius Butenas, Anetta Undas

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Elevated carbonylated proteins are associated with major cardiovascular events in patients with chronic coronary syndrome: A cohort study

Short title: Carbonylated proteins in coronary artery disease

Krystian Mróz[1]*, Elżbieta Paszek[1, 2]*, Mateusz Baran[3], Michał Ząbczyk[2, 4], Saulius Butenas[5], Anetta Undas[2,4]

[1]Clinical Department of Interventional Cardiology, St. John Paul II Hospital, Kraków, Poland
[2]Department of Thromboembolic Disorders, Institute of Cardiology, Jagiellonian University Medical College, Kraków, Poland
[3]AGH University of Krakow, Kraków, Poland
[4]Krakow Center for Medical Research and Technologies, St. John Paul II Hospital, Kraków, Poland
[5]Department of Biochemistry, University of Vermont, Burlington, Vermont, United States
*Both authors equally contributed to the study.

Correspondence to:
Elżbieta Paszek, MD, PhD, MSc,
Department of Thromboembolic Disorders,
Institute of Cardiology,
Jagiellonian University Medical College,
Prądnicka 80, 31–202 Kraków, Poland,
phone: +48 12 614 35 01 ,
e-mail: elzbieta.m.paszek@gmail.com

WHAT'S NEW?

This study shows, for the first time, that increased plasma carbonylated protein levels in patients with advanced coronary artery disease are independently associated with adverse cardiovascular events, including death, in long-term follow-up. We have shown that harmful effects of enhanced protein carbonylation are, at least in part, linked to formation of more compact fibrin clot networks and impaired susceptibility to lysis. Our results provide new insights into the role of posttranslational oxidative modifications in atherothrombosis.

ABSTRACT

Background: Protein carbonylation is reported in atherosclerosis, but its predictive value is unknown.

Aims: We evaluated plasma carbonylated protein (PC) levels in association with clinical outcomes in coronary artery disease (CAD) in long-term follow-up.

Methods: In patients with advanced stable CAD we assessed plasma PC content along with fibrin clot properties, i.e., permeability (K_s) and clot lysis time, and its determinants: plasminogen activator inhibitor-1 (PAI-1) and thrombin-activatable fibrinolysis inhibitor. We recorded a composite of myocardial infarction, ischemic stroke, systemic embolism, and cardiovascular death during a follow-up of 8.3 (1.8) years.

Results: The analysis involved 178 patients aged 64.0 (57.0–70.0) years. The baseline PC content was 2.9 (2.2–3.7) nmol/mg protein and was elevated above the reference value obtained for a control group (2.03 nmol/mg protein) in 82.6% of patients. In linear regression models high PC adjusted for age was associated with lower K_s, longer clot lysis time, along with elevated PAI-1 and thrombin-activatable fibrinolysis inhibitor. Baseline PC was 48% higher in patients with the composite endpoint (n = 67, 37.6%) compared with the remainder (P <0.001). Patients with PC in the highest quartile (3.7–5.1 nmol/mg protein) were more likely to develop the composite endpoint as compared with the lowest quartile (hazard ratio [HR] 4.9; 95% confidence interval, 2.1–11.3; P <0.001).

Conclusions: This is the first study showing that in CAD the extent of protein carbonylation, in part *via* its antifibrinolytic effects, predisposes to cardiovascular events in long-term follow-up, highlighting the role of persistent oxidative protein modifications in atherosclerotic vascular disease.

Key words: carbonylated proteins, cardiovascular death, coronary artery disease, ischemic stroke, myocardial infarction, oxidative stress

INTRODUCTION

Oxidative stress is a common denominator shared by major cardiovascular (CV) risk factors, i.e., hypertension, hypercholesterolemia, diabetes, and smoking [1], and it is involved in coronary microvascular angina [2]. Reactive oxygen species and oxidized polyphenols lead to protein carbonylation (PC), i.e., the introduction of reactive carbonyl groups, i.e., ketone, aldehyde, and lactam into the side chains of amino acids, which might alter the protein structure and function [3]. Such a modification may also be secondary to lipid peroxidation [4]. A study

of 350 subjects showed that advanced oxidation protein products were positively associated with a mean and maximum carotid intima-media thickness [5]. Becatti et al. [6] have shown that in myocardial infarction (MI) survivors assessed 6 months following the index hospitalization, PC was associated with denser fibrin clots and resistance to plasmin-dependent fibrinolysis. In acute ischemic stroke (IS) baseline PC levels were associated with an unfavorable fibrin clot phenotype, and a higher risk of poststroke disability or death assessed 3 months following the acute event [7].

To our knowledge, there have been no studies on a prognostic value of PC in patients with stable coronary artery disease (CAD). We evaluated plasma PC levels and their determinants in stable CAD, and then a prognostic value of this modification during long-term follow-up.

METHODS

Between 2013 and 2015 we enrolled consecutive patients with stable CAD and a ≥50% stenosis in at least 1 major epicardial artery, i.e., >2 mm reference diameter on coronary angiography. The subgroup of the current population was described previously [8]. The exclusion criteria included: acute coronary syndrome 1 month before enrollment or percutaneous coronary intervention within 6 months before enrollment, prior coronary artery bypass grafting, neoplasms, end-stage kidney or liver disease, inflammation, or anticoagulation (Supplementary material, *Figure S1*). Definitions of comorbidities were presented in Supplementary materials. The study protocol conforms to the 1975 Declaration of Helsinki ethical guidelines and was approved by the Jagiellonian University Medical College Ethics Committee. Study participants provided informed written consent.

Laboratory measurements

In fasting venous blood samples complete blood counts, plasma lipid profile, glucose, and creatinine were assayed by standard laboratory techniques. High-sensitive C-reactive protein and fibrinogen were assessed by nephelometry (Siemens, Marburg, Germany). Enzyme-linked immunosorbent assay was used to assess plasminogen activator inhibitor-1 (PAI-1) antigen (Zymutest PAI-1 Antigen, Hyphen BioMed, Neuville-Sur-Oise, France) and thrombin-activatable fibrinolysis inhibitor (TAFI) zymogen (Zymutest Activable TAFI, Hyphen BioMed), which is presented as a percentage of a pooled standard plasma. 8-iso-prostaglandin F2α (8-iso-PGF2α) was assessed immunoenzymatically (Cayman Chemicals, Ann Arbor, MI, US).

Fibrin clot analysis

Fibrin clot permeation was assessed as described previously [9]. In short, we mixed 60 µl of a coagulation trigger containing 1 IU/ml human thrombin, 20 mM $CaCl_2$ and 60 µl of citrated plasma to generate a clot in a plastic cylinder (Sarstedt, Nümbrecht, Germany). We measured the volume of the percolated buffer. We calculated the permeability coefficient (K_s), as a measure of the average pore size in the fiber network, using the equation: K_s ($\times 10^{-9}$ cm^2) = Q × L × η/t × A × ΔP, where Q (cm^3) is the flow rate at time t (s), L (cm) is the length of the fibrin gel, η (dyne × s/cm^2) is the viscosity of the liquid, A (cm^2) is the cross-sectional area and Δp (dyne/cm^2) is differential pressure. In our laboratory the upper reference K_s value in healthy patients is 7.4×10^{-9} cm^2. The inter- and intra-assay variability were <7%.

Clot lysis time (CLT) was assessed, as previously described [9]. Briefly, we added 15 mmol/l $CaCl_2$, 0.6 pM human tissue factor (Innovin, Siemens), 12 µmol/l phospholipid vesicles, and 60 ng/ml recombined tissue plasminogen activator (rtPA, Boehringer Ingelheim, Ingelheim, Germany) to 100 µl of citrated plasma. We measured absorbance at 405 nm in 37°C (Tecan Sunrise). We defined CLT as the time from clot formation to lysis, i.e., midpoint of the clear-to-maximum-turbid transition to the midpoint of the maximum-turbid-to-clear transition. The upper reference value for CLT in healthy subjects at our laboratory is 84 min. The inter- and intra-assay variability were <6%.

Carbonylation measurement

Carbonyl content was measured using a method by Becatti et al [6]. Briefly, 400 µl DNPH was added to 100 µl of plasma. Following incubation, trichloracetic acid was added for precipitation. The pellet was washed with a 1:1 solution of ethanol/ethyl acetate and resuspended in 500 µl of guanidine hydrochloride. PC content was normalized for total protein concentration and expressed as nmol/mg of protein. The reference values for apparently healthy controls, aged 56 (39–64) years, 70% male, were 0.54–2.03 nmol/mg. A detailed characteristics of the control group can be found in Supplementary material, *Table S1*. A similar reference value was reported for healthy volunteers by Becatti et al. [6]. The inter- and intraassay variability of the results is <10%.

Follow-up

Data was censored in January 2023. The primary endpoint was a composite of MI, IS, systemic thromboembolism (SE), and CV death. Secondary endpoints were MI, IS/SE, and CV death analyzed separately. We did not record type 2 MI. CV mortality was coded when the cause of

death were: MI, IS, thromboembolism of any other vascular bed, heart failure, arrhythmia or a CV procedure. Follow-up was conducted during a clinic visit or by a phone call. We asked patients or their families to provide medical records for confirmation. We also used the National Mortality Registry maintained by the State Systems Department of Ministry of Digital Affairs to assess patient status.

Statistical analysis

We assumed the rate of the composite endpoint at 15% for patients with PC in the bottom quartile and 60% for patients in the top quartile, based on data from large registries [10, 11]. The estimated hazard ratio (HR) was 4.0, the level of significance was set at 0.05 and the power of the test at 90%, which led to the overall number of participants of 156, 39 patients per quartile.

The Shapiro–Wilk test was used for assessment of continuous variables distribution. Continuous data were presented as mean (standard deviation) or as medians (Q1–Q3). Continuous variables were compared using the t-Student test or U Mann–Whitney test, as appropriate in case of 2 groups and ANOVA or Kruskal–Wallis when more than 2 groups were compared. *Post hoc* Dunn's or Tukey's tests were used, as appropriate. Categorical data were presented as numbers (percentages), and Fisher's exact test was used to compare them. Spearmann's correlation was used to check for associations between continuous variables. The effects of variables on the endpoints were evaluated using Cox proportional hazard models. Results were presented as HR with 95% confidence intervals (CI). CIs for area under the curve scores were calculated using DeLong's method. Kaplan–Meier survival curves were plotted. Cox proportional hazard models were used to find endpoint predictors. Multivariable Cox proportional hazard models were used to evaluate HR adjusted for confounders. We chose possible confounders from parameters which differed between patients with and without the endpoints based on results of ANOVA or Kruskal–Wallis tests; we included age and sex as additional confounders. Akaike information criterion was used for selection of best models. We established optimal cut-off points for PC values predicting endpoints based the Youden's J statistic. Statistical analyses were performed using Python and R libraries. A level of significance of 0.01 was used for the normality of data distribution and assumptions for the Cox analysis. Otherwise a level of significance <0.05 was considered statistically significant.

RESULTS

We analyzed 178 patients, aged 64.0 (57.0–70.0) years, 75.8% male (Table 1). Two patients (1.1%) were excluded due to C-reactive protein >100 mg/l. Most of the patients (n = 132, 74.2%)

had multivessel disease, while 127 (71.3%) had a history of MI or percutaneous coronary intervention (Table 1).

The median PC content in the cohort was 2.9 (2.2–3.7) nmol/mg protein, while 147 (82.6%) patients had PC above the upper reference limit (>2.03 nmol/mg protein). There were no differences in clinical, demographic, or routine laboratory parameters between patients with PC in different quartiles, with the exception of age, which associated positively with PC (r = 0.50; *P* <0.001; Supplementary material, *Figure S2*). PC levels correlated positively with 8-iso-PGF2α (r = 0.33; *P* <0.001).

Patients with PC in the highest quartile had lower K_s and longer CLT, compared with those in the lowest one (Table 1). Subjects in the top PC quartile had higher PAI-1 and TAFI than those in the bottom quartile (Table 1). PC levels were inversely associated with K_s (r = –0.26; *P* <0.001; Figure 1A) and positively with CLT (r = 0.30; *P* < 0.001; Figure 1B), along with its determinants, i.e. PAI-1 (r = 0.33; *P* <0.001; Figure 1C) and TAFI (r = 0.32; *P* <0.001; Figure 1D).

Follow-up

During a follow-up of 8.3 (1.8) years the composite endpoint occurred in 67 (37.6%) patients, (0.43 per 100 patient-years), including 35 with MI, 25 with IS + SE and 30 with CV death. Eight patients experienced MI and CV death, 12 patients had IS and died of CV causes, 2 had SE and died of CV causes, and 1 patient experienced IS, SE and CV death (Supplementary material, *Figure S1*). Patients with and without the composite endpoint were similar, except for higher body mass index and hypertension prevalence in the former group (Table 2).

Patients with the composite endpoint had lower K_s and longer CLT, along with higher TAFI. PC levels were 48% higher in the group with the composite endpoint (Table 2). After adjustment for potential confounders, PC in the highest quartile remained a predictor of the composite endpoint (HR, 4.89; 95% CI, 2.12–11.27; *P* <0.001; Figure 2A). An increase of PC by 1 nmol/mg was associated with a 2.2-fold higher risk of the composite endpoint on multivariable analysis (Supplementary material, *Table S3*).

We recorded MI in 35 (19.7%) patients, 0.20 per 100 patient-years. The PC content in this group was 38.5% higher as compared with the remainder (Supplementary material, T*able S2*). After adjustment for age, sex, and LDL-C, PC in the highest quartile was associated with higher MI risk (HR, 6.25; 95% CI, 1.70–23.01; *P* = 0.006; Figure 2B). On multivariable analysis PC remained an independent predictor of MI (Supplementary material, *Table S3*).

Twenty-five (14.0%) patients experienced IS/SE (0.14 per 100 patient-years). Baseline PC were 44.4% higher in patients with an IS/SE than in the remainder (Supplementary material, *Table S2*). After adjustment for age and sex, PC in the top quartile was associated with the risk of IS/SE when compared to the bottom quartile (HR, 21.37; 95% CI, 2.67–170.96; $P = 0.004$; Figure 2C) and this association remained significant on multivariable analysis (Supplementary material, *Table S3*).

Thirty patients (16.9%) died of CV causes (0.17 per 100 patient-years). PC content and prolonged CLT distinguished these patients from the remainder (Supplementary material, *Table S2*). After adjustment for age and sex, PC in the highest quartile was associated with the risk of death from CV causes (HR, 4.87; 95% CI, 1.46–26.21; $P = 0.01$; Figure 2D). On multivariable analysis PC remained independently associated with CV mortality (Supplementary material, *Table S3*).

Based on ROC curves (Supplementary material, *Figure S3*), the optimal cut-off value for baseline PC was 3.03 nmol/mg protein with an area under the curve of 0.753; 95% CI, 0.678–0.827 for the composite endpoint.

DISCUSSION

This study is the first to show that in patients with stable CAD high plasma PC content can predict MI, IS, SE, and CV death, analyzed separately and as a composite endpoint, in long-term follow-up. The composite endpoint was mostly driven by MI, however elevated PC had the largest impact on the risk of IS/SE, with a 3.8-fold risk increase per 1 nmol/mg protein. Our results suggest that persistent protein carbonylation contributes to a prothrombotic state in CAD. A novel finding in stable CAD is that the extent of protein carbonylation is associated with low clot permeability and prolonged CLT, along with elevated concentrations of PAI-1 and TAFI, which indicates prothrombotic and antifibrinolytic effects of this protein modification. Our study provides additional evidence that in advanced CAD complex mechanisms governing carbonylation persist and predispose to CV events, largely thromboembolic by nature.

Our population was similar to those reported in large registries [10, 11]. In the seminal study by Becatti et al. [6], patients aged 71 (59–77) years, 69% men, 6 months after an MI, had PC levels of 2.87 (1.02) nmol/mg [5]. This mean value was almost identical to the median of 2.9 (2.2–3.7) nmol/mg protein in our cohort with comparable demographics. In our study PC content correlated with age, which is consistent with previous data [12]. Redox imbalance has been implicated in a number of atherosclerosis-related diseases such as hypertension [13], hypercholesterolemia [14], diabetes [15], and obstructive sleep apnea [16]. We found no such

associations, which is most likely attributable to the advancement of CAD in our group, with over 74% of patients with multivessel disease, which implies (*via* the abundant atherosclerosis burden) a substantial reactive oxygen species generation.

Mechanisms leading to a persistent presence of circulating PC in CAD are largely unknown. Free radicals generated in a low-grade inflammatory state typical of CAD can cause carbonylation. It might be speculated that in atherosclerosis protein carbonylation is the most suitable marker of the detrimental impact of oxidative stress on circulating proteins contributing to cardiovascular events.

We have provided additional evidence that elevated PC enhance a prothrombotic state in CAD. We observed altered fibrin clot properties including decreased permeability and resistance to lysis in association with PC, and such a prothrombotic clot phenotype was reported in chronic and acute coronary syndromes [17]. Since fibrinogen concentration and function are key determinants of fibrin clot properties [18], it might be speculated that enhanced fibrinogen carbonylation largely contributes to the prothrombotic clot phenotype in plasma-based assays. Paton et al. have shown that in patients with MI who had PC in the top quartile, fibrinogen polymerization was 1.4-fold faster and gave 1.4 times higher maximum turbidity as compared with those in the bottom quartile, reflecting faster lateral fibrin aggregation [18]. It is likely that similar reactions though of lesser intensity, could be observed in stable CAD, since fibrinogen is particularly susceptible to oxidative modifications [19]. Fibrinogen carbonylation contributes to the formation of fibrin clots which are resistant to tissue plasminogen activator-induced lysis, an effect attributable to lysine carbonylation and a subsequent modification of the binding sequence for plasminogen [20]. Such impaired plasminogen-fibrin interactions in enhanced PC have been demonstrated in post-VTE patients [21]. As summarized by de Vries et al. [19], the effects of fibrinogen oxidation on clot properties *in vitro* vary depending on the type of oxidation trigger used and the concentrations of oxidants.

In the current study elevated PC was weakly associated with higher PAI-1 and TAFI, well-established modulators of clot lysability [22] and with longer CLT, which represents impaired global fibrinolysis. Similar associations have been recently reported in acute IS [7]. Oxidative stress was linked to elevated PAI-1 expression [23]. Moreover, lysine carbonylation could decrease the number of cleavage sites for both plasmin and tissue-type plasminogen activator [24], leading to impaired fibrinolysis.

The reasons for a rise in TAFI with elevated PC are unclear. TAFI circulates in the plasma bound to plasminogen and is activated by the thrombin/thrombomodulin complex, which cleaves TAFI on Arg92 [25]. Since arginine is prone to carbonylation, carbonylation

could alter the cleavage site for thrombin and facilitate its dissociation from plasminogen, which would lead to a higher detectable concentration. Our unexpected observations deserve further mechanistic studies.

A positive correlation between PC and 8-iso-PGF2α confirms that both these markers reflect enhanced oxidative stress in advanced CAD. Protein carbonylation represents redox imbalance over longer periods of time, while isoprostanes reflect short-term effects.

The current study has several limitations. Firstly, the sample size was relatively small, however sufficient to show the assumed effect, according to power calculation. The patients in this study used similar pharmacotherapy, with almost 90% on statins, which did not allow for an analysis of these drugs as potential confounders. Also, modifications of pharmacotherapy during the follow-up period could affect the results. The mechanistic basis for the observed associations between PC levels and impaired fibrinolysis were not investigated in the current study. We have, however, shown positive correlations between PC and PAI-1, as well as TAFI, which are 2 key antifibrinolytic proteins. In line, an association between PC and impaired fibrinolysis was reported for MI and IS survivors [6, 7]. We did not determine carbonylated fibrinogen, however a positive association between overall and fibrinogen carbonylation has already been documented [6].

To conclude, this study shows a novel, independent association between plasma PC content, a stable marker of oxidative stress, and the risk of atherothrombotic events in stable CAD, likely linked to prothrombotic alterations of the fibrin clot phenotype. It is likely that the current management of advanced CAD cannot substantially reduce these processes, therefore plasma PC concentrations could be perceived as a specific type of “residual risk” to be treated differently.

Supplementary material

Supplementary material is available at https://journals.viamedica.pl/polish_heart_journal.

Article information

Conflict of interest: None declared.

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Table 1. Patient characteristics according to quartiles of plasma carbonylated protein content

		Carbonylated protein content, nmol/mg				
	Whole group (n = 178)	Q1 (1.3–2.2)	Q2 (2.2–2.9)	Q3 (2.9–3.1)	Q4 (3.1-5.1)	*P*-value
Age, years	64 (57–70)	57 (53–64)	63 (57–68)	65 (60–69)	73 (64–73)	**<0.001**[a]
Male, n (%)	135 (75.8)	35 (81.4)	30 (66.7)	37 (86.1)	31 (68.9)	0.10
BMI, kg/m^2	26.9 (3.9)	26.3 (4.1)	27.1 (4.1)	26.8 (3.6)	27.5 (3.6)	0.54
Comorbidities, n (%)						
Smoking	57 (32.0)	19 (44.2)	14 (31.1)	11 (25.6)	13 (28.9)	0.27
Diabetes	36 (20.2)	8 (18.6)	10 (22.2)	9 (20.9)	9 (20.0)	0.98
Hypertension	133 (74.7)	31 (72.1)	33 (73.3)	32 (74.4)	35 (77.8)	0.94
Prior MI/PCI	127 (71.3)	32 (74.4)	30 (66.7)	30 (69.8)	33 (73.3)	0.85

Medications, n (%)						
ACE-I/ARB	125 (70.2)	32 (74.4)	30 (66.7)	26 (60.5)	35 (77.8)	0.29
Statins	156 (87.6)	41 (95.4)	40 (88.9)	36 (83.7)	37 (82.2)	0.24
Laboratory parameters						
WBC, 10^3/µl	6.6 (5.5–8.3)	6.6 (5.3–8.1)	7.1 (6.4–8.6)	6.1 (4.9–7.4)	6.4 (5.5–7.9)	0.06
Hemoglobin, g/dl	13.6 (1.4)	13.5 (1.3)	13.8 (1.5)	13.7 (1.3)	13.6 (1.5)	0.77
Creatinine, µmol/l	79 (66–90)	82 (67–89)	84 (67–95)	73 (63–87)	76 (67–85)	0.25
TC, mmol/l	4.4 (3.7–5.3)	4.6 (3.7–5.7)	4.1 (3.6–4.7)	4.3 (3.6–5.3)	4.5 (3.8–5.2)	0.42
LDL-C, mmol/l	2.5 (1.9–3.4)	2.9 (1.9–3.6)	2.5 (2.1–3.1)	2.5 (2.0–3.4)	2.7 (1.9–3.4)	0.79
HDL-C, mmol/l	1.2 (1.0–1.4)	1.3 (1.1–1.4)	1.2 (1.1–1.3)	1.1 (1.0–1.3)	1.2 (1.0–1.3)	0.38
Glucose, mmol/l	5.3 (5.0–5.9)	5.2 (5.0–5.8)	5.5 (5.0–6.0)	5.4 (5.1–6.0)	5.2 (4.9–5.6)	0.42
Fibrinogen, g/l	3.3 (2.6–4.3)	3.1 (2.5–4.1)	3.2 (2.8–3.8)	3.5 (2.7–4.6)	3.5 (2.8–4.7)	0.57
8-iso-PGF2α, pg/ml	346 (279–423)	297 (251–330)	323 (280–367)	395 (321–458)	376 (279–455)	**<0.001**[c]
Fibrin clot properties and associated proteins						
K_s, 10^{-9} cm^2	6.6 (0.9)	7.1 (0.9)	6.5 (0.9)	6.4 (0.8)	6.4 (1.0)	**<0.001**[a]
CLT, min	103.3 (18.0)	92.0 (13.8)	103.3 (13.7)	107.45 (16.8)	111.1 (20.2)	**<0.001**[a]
TAFI, %	100 (91–111)	96 (86–103)	100 (91–107)	100 (94–113)	106 (96–118)	**0.005**[b]
PAI-1, ng/ml	51.1 (13.0)	43.3 (11.9)	51.4 (11.3)	54.6 (11.4)	55.4 (13.1)	**<0.001**[a]

Continuous data were shown as mean (SD) or median (Q1–Q3)

[a]*Post hoc* test: significant differences: Q1 vs. Q2, Q1 vs. Q3 and Q1 vs. Q4. [b]*Post hoc* analysis: significant differences: Q1 vs. Q3 and Q1 vs. Q4. [c]*Post hoc* analysis: significant differences: Q1 vs. Q3, Q1 vs. Q4 and Q2 vs. Q3

Abbreviations: 8-iso-PGF2α, 8-iso-prostaglandin F2α; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blockers; BMI, body mass index; CLT, clot lysis time; HDL-C, high-density lipoprotein cholesterol; K_s, permeation coefficient; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; PAI-1, plasminogen activator inhibitor-1; PCI, percutaneous coronary intervention; TAFI, thrombin-activatable fibrinolysis inhibitor; TC, total cholesterol; WBC, white blood cells

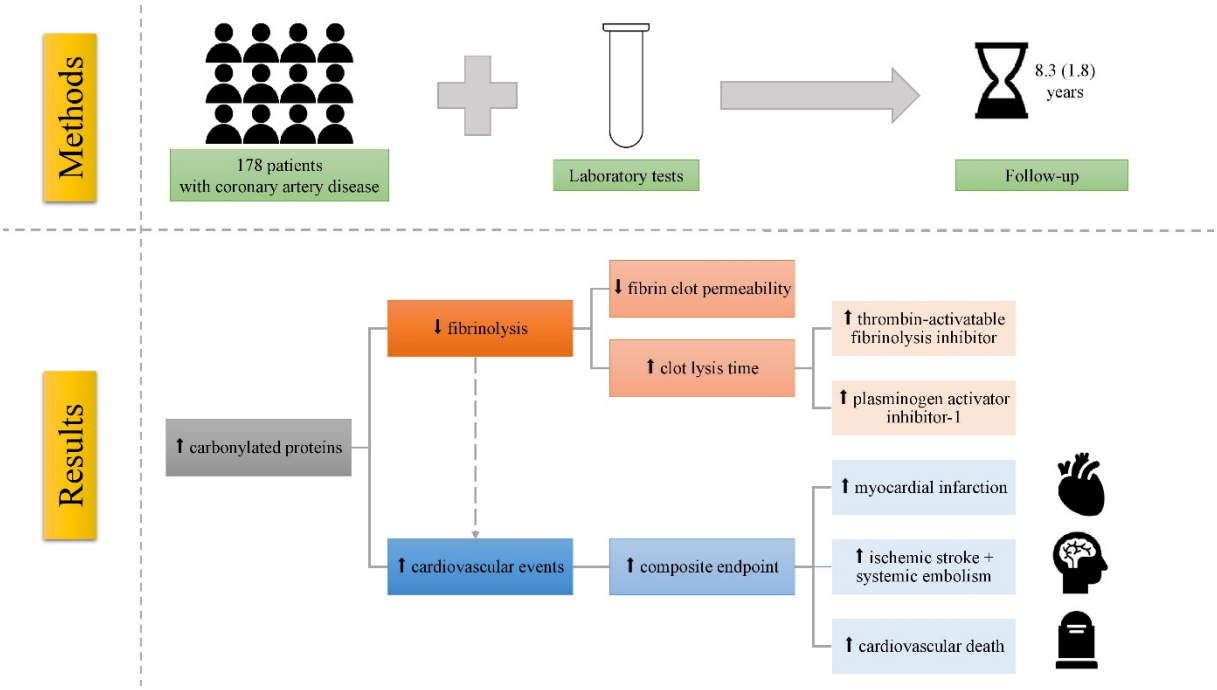
Table 2. Patient characteristics based on the occurrence of the composite endpoint during follow-up

	Composite endpoint		
	Yes	No	*P*-value

	(n = 67)	(n = 111)	
Age, years	66 (57–73)	63 (56–69)	0.08
Male, n (%)	53 (79.1)	82 (73.9)	0.43
BMI, kg/m^2	27.9 (3.5)	26.4 (4.0)	**0.009**
Smoking, n (%)	21 (31.3)	36 (32.4)	0.88
Comorbidities, n (%)			
Diabetes, n (%)	14 (20.9)	22 (19.8)	0.83
Hypertension, n (%)	56 (83.6)	77 (69.4)	**0.03**
Prior MI or PCI, n (%)	52 (77.6)	75 (67.6)	0.15
Medications, n (%)			
ACE-I, n (%)	51 (76.1)	74 (66.7)	0.18
Statins, n (%)	55 (82.1)	101 (91.0)	0.08
Laboratory parameters			
White blood cells, $10^3/\mu l$	6.7 (5.3–8.5)	6.5 (5.5–8.1)	0.64
Hemoglobin, g/dl	13.8 (12.7–14.8)	13.8 (12.5–14.4)	0.37
Creatinine, µmol/l	76.2 (65.2–94.0)	79.1 (66.0–89.1)	0.80
CRP, mg/l	2.2 (1.4–3.6)	2.0 (1.2–3.5)	0.49
TC, mmol/l	4.8 (4.0–5.8)	4.2 (3.4–5.0)	**0.001**
LDL-C, mmol/l	2.9 (2.1–3.8)	2.4 (1.9–3.2)	**0.013**
HDL-C, mmol/l	1.2 (1.0–1.4)	1.2 (1.0–1.3)	0.29
Glucose, mmol/l	5.3 (4.9–6.0)	5.3 (5.0–5.8)	0.54
Fibrinogen, g/l	3.3 (2.5–4.5)	3.2 (2.7–4.3)	0.87
Fibrin clot properties and associated proteins			
K_s, 10^{-9} cm^2	6.4 (0.9)	6.8 (1.0)	**0.009**
CLT, min	108 (100–127)	99 (89–107)	**<0.001**
TAFIAg, %	103.9 (96.7–113)	100 (87–110)	**0.023**
PAI-1, ng/ml	52.9 (13.6)	49.9 (12.5)	0.14
Total PC content, nmol/mg protein	3.7 (2.9–4.1)	2.5(2.1–3.2)	**<0.001**

Continuous data were shown as mean (SD) or median (Q1–Q3)

Abbreviations: CRP, C-reactive protein; thrombin-activatable fibrinolysis inhibitor antigen; other — see Table 1



Graphical abstract. Protein carbonylation in coronary artery disease

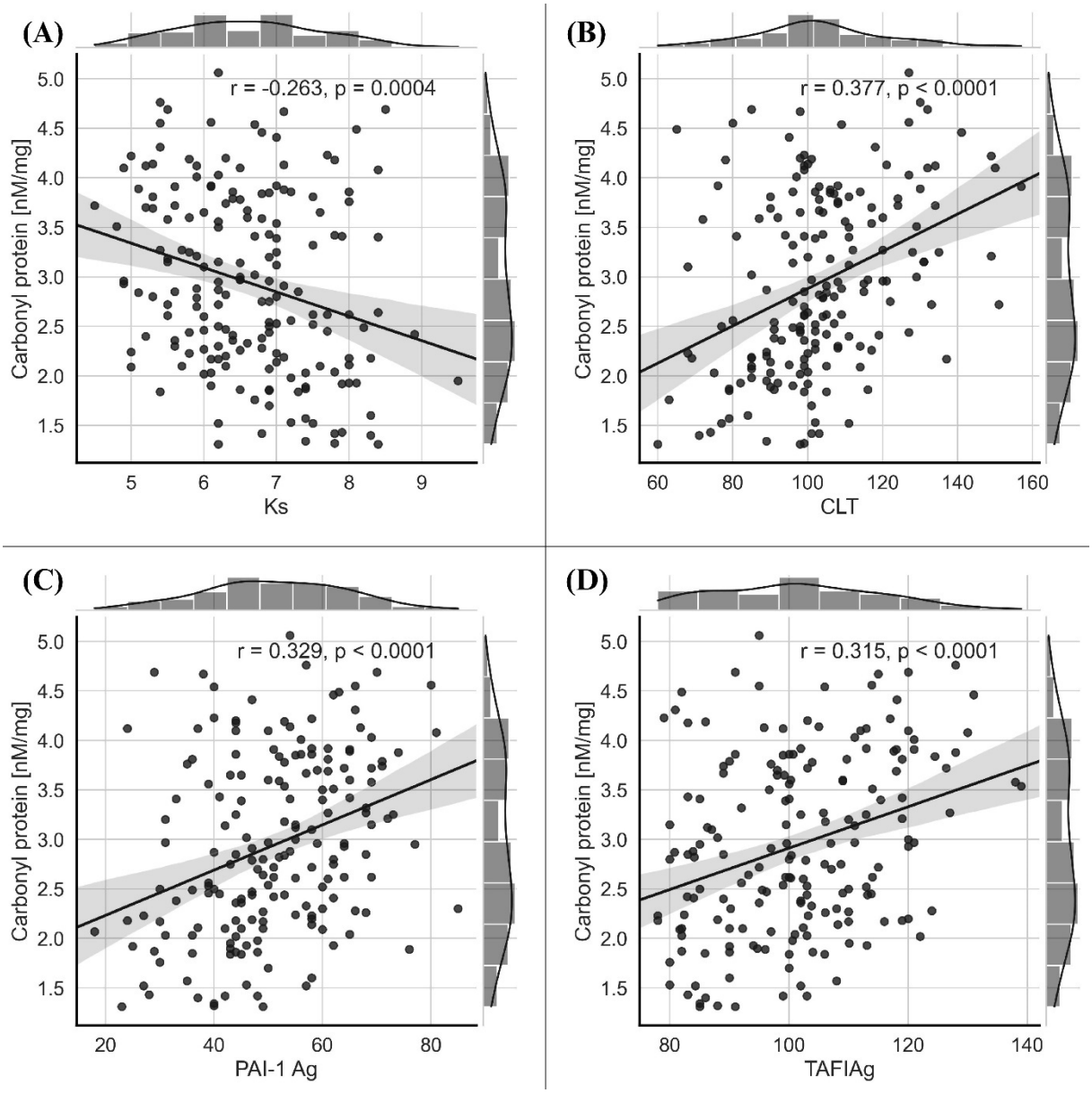


Figure 1. Correlation charts with regression lines, 95% confidence intervals. Association between carbonyl protein content and **A.** Permeation coefficient (K_s). **B.** Clot lysis time (CLT). **C.** Plasminogen activator inhibitor 1 antigen (PAI-1 Ag). **D.** Thrombin-activatable fibrinolysis inhibitor antigen (TAFIAg)

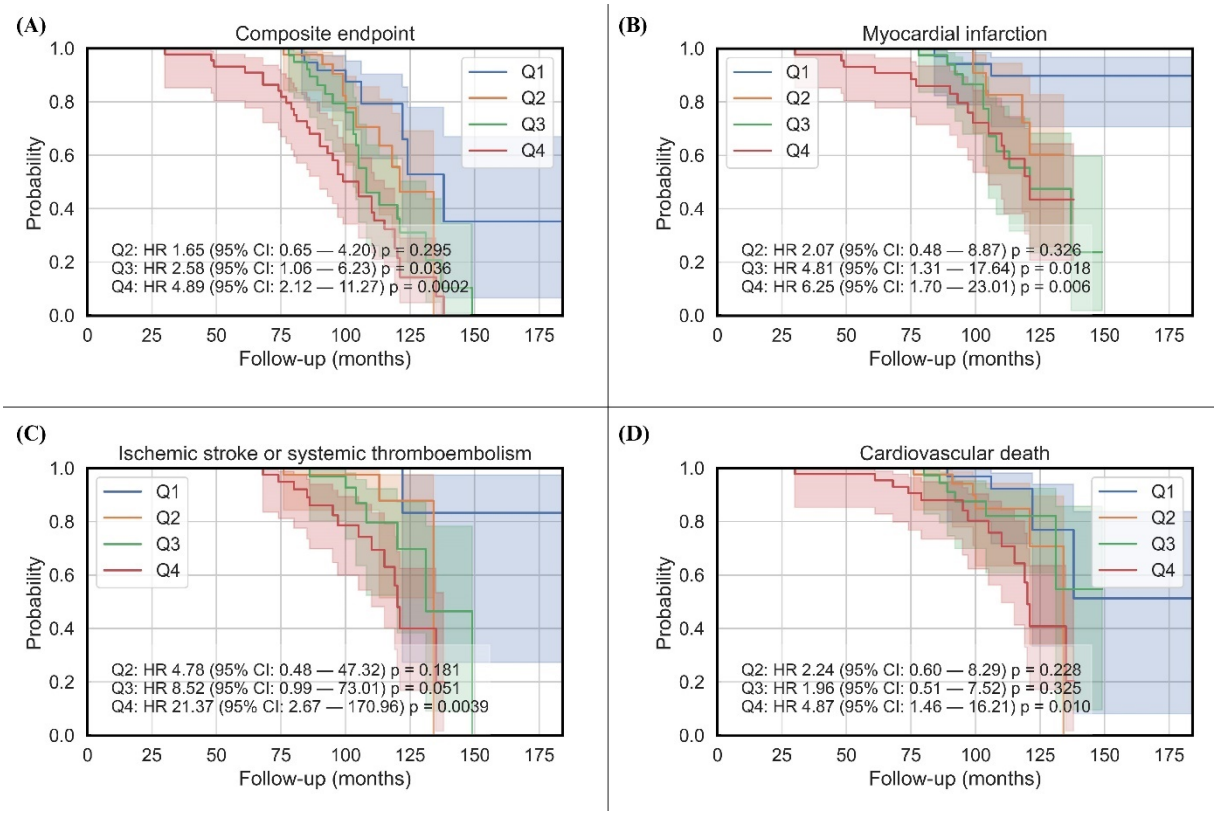


Figure 2. Kaplan–Meier curves showing the probability of event-free survival with regard to carbonylated protein content, after adjustment for potential confounders, in patients with coronary artery disease

Abbreviations: CI, confidence interval; HR, hazard ratio