Supplementary material

Mróz K, Paszek E, Baran M, et al. Elevated carbonylated proteins are associated with major cardiovascular events in patients with chronic coronary syndrome: A cohort study. Pol Heart J. 2024.

Definitions of comorbidities for the studied cohort

Hypertension was diagnosed in case of blood pressure ≥140/90 mm Hg measured on two separate occasions, taking antihypertensive drugs or a history of hypertension. Hypercholesterolemia was defined at the presence of total cholesterol >5.0 mmol/l or low-density lipoprotein cholesterol >3.0 mmol/l, or the use of statins. If fasting glycemia was above 7.0 mmol/l (126 mg/dl), or a patient had been diagnosed before, diabetes was coded.

Table S1. Characteristics of patients and controls. Values were shown as mean (standard deviation) or median (Q1–Q3)

Variable	CAD patients, n = 178	Healthy control, n = 30	*P*-value
Age, years	64 (57–70)	56 (39–64)	<0.001
Male, n (%)	135 (75.8)	21 (70)	0.31
Body mass index, kg/m^2	27.0 (3.9)	25 (2.9)	0.002
Comorbidities and medications, n (%)			
Diabetes	36 (20.2)	0	
Hypertension	133 (74.7)	0	
Prior MI/PCI	127 (71.3)	0	
ACE-I	125 (70.2)	0	
Statins	156 (87.6)	0	
Laboratory investigations			
WBC, 10^3/µl	6.6 (5.5–8.3)	6.1 (5.3–8.0)	0.14
Hemoglobin, g/dl	13.8 (12.6–14.5)	14.1 (13.1–15.3)	0.34

Creatinine, μmol/l	78.9 (66.0–90.0)	69 (64.0–75.0)	0.008
Total cholesterol, mg/dl	4.4 (3.7–5.3)	5.2 (4.8–6.2)	<0.001
LDL-C, mmol/l	2.5 (1.9–3.4)	3.7 (2.9–4.1)	<0.001
HDL-C, mmol/l	1.2 (1.0–1.4)	1.7 (1.4–1.9)	<0.001
Triglycerides, mmol/l	1.4 (1.0–1.9)	1.2 (0.9–1.6)	0.09
Glucose, mmol/l	5.3 (5.0–5.9)	5.4 (5.1–5.6)	0.70
Fibrinogen, g/l	3.2 (2.6–4.3)	3.0 (2.9–3.4)	0.02
8-iso-PGF2α, pg/ml	346.5 (89.0)	46.0 (13.0)	<0.001
K_s, $\times 10^{-9} cm^2$	6.6 (1.0)	7.8 (7.1–8.3)	<0.001
CLT, min	102 (92–112)	85 (74–98)	<0.001
TAFI, %	100.5 (21.0)	89.0 (11.0)	<0.001
PAI-1, ng/ml	51.1 (13.0)	15.0 (7.9–19.5)	<0.001

Abbreviations: 8-iso-PGF2α, 8-iso-prostaglandin F2α; ACE-I, angiotensin-converting enzyme inhibitor; CAD, coronary artery disease; 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; WBC, white blood cells

Table S2. Patient characteristics based on the occurrence of single clinical endpoints during follow-up. Values were shown as mean (standard deviation) or median (Q1–Q3)

Variable	Myocardial infarction			Ischemic stroke or systemic embolism			Cardiovascular death		
	Yes (n = 35)	No (n = 143)	*P*-value	Yes (n = 25)	No (n = 153)	*P*-value	Yes (n = 30)	No (n = 148)	*P*-value
Age, years	67 (59–73)	64 (56–70)	0.13	65 (58–74)	63 (57–70)	0.35	64 (56–74)	64 (57–70)	0.61
Male, n (%)	27 (77.1)	108 (75.5)	0.84	21 (84.0)	114 (74.5)	0.22	24 (80)	111 (75)	0.55
BMI, kg/m^2	27.2 (3.5)	26.9 (4.0)	0.70	29.1 (26.4–31.5)	26.6 (23.8–29.1)	**0.004**	27.9 (3.5)	26.8 (3.9)	0.14
Smoking, n (%)	7 (20.0)	50 (35.0)	0.08	10 (40.0)	47 (31.0)	0.24	10 (33.0)	47 (32.0)	0.87
Diabetes, n (%)	7 (20.0)	29 (20.3)	0.97	7 (28.0)	29 (19.0)	0.21	4 (13.0)	32 (22.0)	0.30
Hypertension, n (%)	31 (88.6)	102 (71.3)	**0.03**	20 (80.0)	113 (74.0)	0.35	23 (77.0)	110 (74.0)	0.79
Prior MI or PCI, n (%)	30 (85.7)	97 (67.8)	**0.03**	18 (72.0)	109 (71.0)	0.57	21 (70.0)	106 (72.0)	0.86
Medication, n (%)									
ACE-I, n (%)	29 (82.9)	96 (67.1)	0.06	18 (72.0)	107 (70.0)	0.52	19 (63.0)	106 (72.0)	0.37
Statins, n (%)	30 (85.7)	126 (88.1)	0.70	20 (80.0)	136 (89.0)	0.17	24 (80.0)	132 (89.0)	0.16
Laboratory parameters									
White blood cells, $10^3/\mu l$	6.8 (5.2–8.9)	6.6 (5.5–8.1)	0.78	6.8 (5.7–8.3)	6.6 (5.5–8.2)	0.51	6.3 (5.3–8.5)	6.6 (5.5–8.2)	0.76
Hemoglobin, g/dl	13.9	13.8	0.68	13.7	13.8	0.54	14.3	13.7	0.11

	(12.6–14.7)	(12.6–14.5)		(12.7–14.8)	(12.6–14.5)		(12.9–14.9)	(12.5–14.4)	
Creatinine, μmol/l	80.2 (60.9–96.0)	78.9 (66.5–89.1)	0.86	75.7 (67.6–85.0)	79.1 (66.0–90.4)	0.48	77.7 (70.2–95.0)	79 (65.0–89.1)	0.30
CRP, mg/l	1.8 (1.1–2.4)	2.0 (1.0–3.8)	0.09	2.3 (1.6–3.6)	2.0 (1.3–3.6)	0.49	2.1 (1.4–4.1)	2.0 (1.2–3.3)	0.62
TC, mmol/l	4.8 (3.9–6.1)	4.3 (3.5–5.3)	**0.022**	4.7 (4.2–5.4)	4.2 (3.6–5.3)	0.15	4.8 (3.8–5.9)	4.3 (3.6–5.2)	0.12
LDL-C, mmol/l	3.0 (2.2–4.1)	2.5 (1.9–3.3)	**0.009**	2.8 (2.3–3.5)	2.5 (1.9–3.3)	0.51	2.7 (1.9–3.5)	2.5 (2.0–3.3)	0.90
HDL-C, mmol/l	1.3 (1.0–1.4)	1.2 (1.0–1.4)	0.50	1.2 (1.1–1.4)	1.2 (1.0–1.3)	0.74	1.2 (1.1–1.3)	1.2 (1.0–1.4)	0.17
Glucose, mmol/l	5.5 (4.9–6.1)	5.3 (5.0–5.8)	0.62	5.0 (4.8–5.6)	5.3 (5.0–5.9)	**0.048**	5.3 (4.9–5.9)	5.3 (5.0–5.9)	0.82
Fibrinogen, g/l	3.4 (2.4–4.8)	3.2 (2.7–4.3)	0.83	3.2 (2.6–4.5)	3.3 (2.6–4.3)	0.64	3.1 (2.5–3.9)	3.3 (2.6–4.5)	0.34
K_s, 10^{-9} cm^2	6.3 (0.8)	6.7 (1.0)	**0.026**	6.2 (5.4–7.1)	6.6 (6.0–7.3)	0.20	6.5 (0.9)	6.6 (1.0)	0.51
CLT, min	109 (102–127)	99 (90–109)	**<0.001**	108 (104–127)	100 (90–111)	**<0.001**	108.5 (99.0–127.0)	100 (90.5–111.0)	**0.007**

TAFIAg, %	104 (92–111)	100 (90–111)	0.19	106 (97–114)	100.3 (90–110)	0.13	103 (98.0–113.9)	100 (89.5–110.0)	0.18
PAI-1, ng/ml	53.7 (13.4)	50.4 (12.8)	0.18	57 (44–64)	50 (43–60)	0.36	53.3 (14.3)	50.6 (12.7)	0.30
Total PC content, nmol/mg protein	3.6 (3.0–3.9)	2.6 (2.1–3.6)	**0.001**	3.9 (3.3–4.1)	2.7 (2.2–3.6)	**<0.001**	3.7 (2.6–4.1)	2.8 (2.2–3.6)	**0.003**

Abbreviations: CRP, C-reactive protein; PC, protein carbonylation; other — see *Table S1*

Table S3. Multivariable analysis using Cox proportional hazards model. 2-sided significance tests were performed for each variable

Variable	Hazard ratio (95% confidence interval)	*P*-value
Composite endpoint		
Carbonylated protein, per 1 nmol/mg protein	2.15 (1.57–2.94)	<0.001
Age, per year	0.98 (0.95–1.01)	0.13
Sex, male	1.05 (0.58–1.90)	0.87
LDL-C, per 1 mmol/l	1.01 (0.80–1.26)	0.95
CLT, per 10 min	1.00 (0.90–1.02)	0.58
K_s, per 10^{-9} cm^2	0.93 (0.69–1.25)	0.63
Myocardial infarction		
Carbonylated protein, per 1 nmol/mg protein	2.08 (1.35–3.23)	0.001
Age, per year	0.99 (0.95–1.04)	0.73
Sex, male	0.93 (0.42–2.05)	0.85
LDL-C, per 1 mmol/l	1.30 (0.97–1.74)	0.08
Ischemic stroke or systemic thromboembolism		
Carbonylated protein, per 1 nmol/mg protein	3.81 (2.06–7.05)	<0.001
Age, per year	0.98 (0.93–1.02)	0.32
Sex, male	1.52 (0.51–4.55)	0.45
Cardiovascular death		
Carbonylated protein, per 1 nmol/mg protein	2.19 (1.36–3.52)	0.001
Age, per year	0.98 (0.94–1.02)	0.34
Sex, male	1.02 (0.41–2.55)	0.96

Abbreviations: see *Table S1*

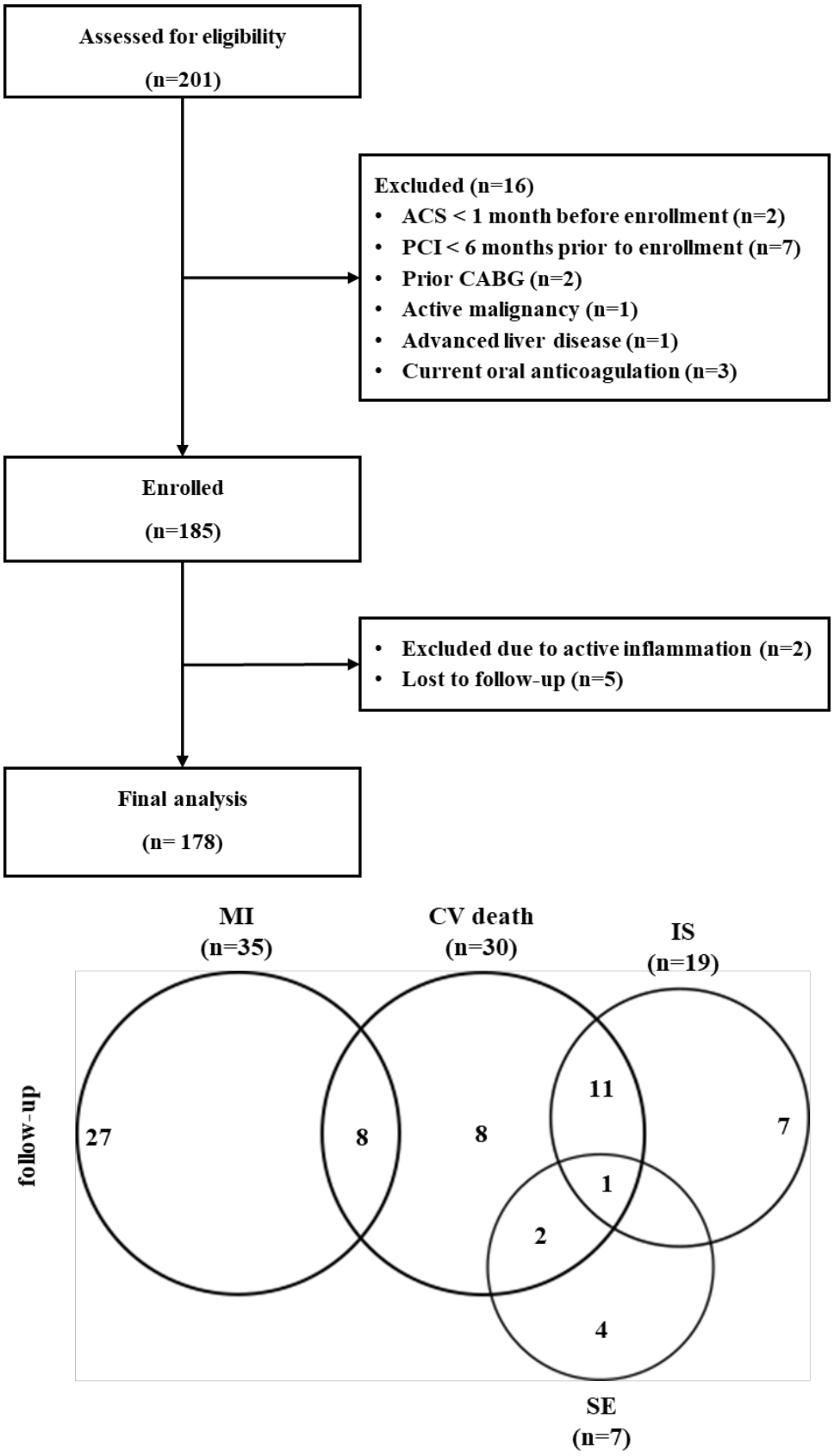

Figure S1. Flow diagram showing the study population

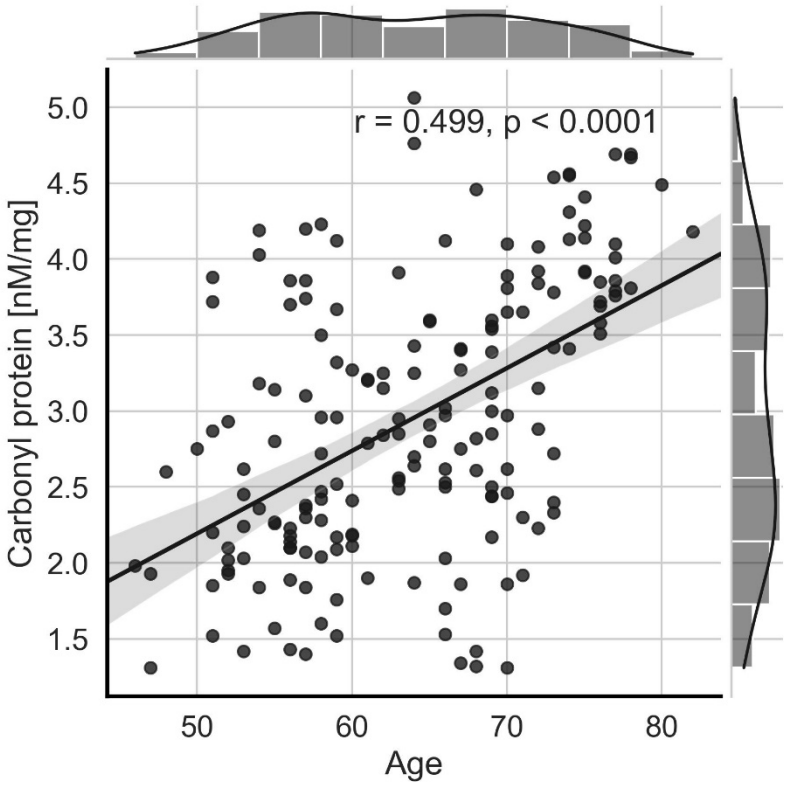

Figure S2. Correlation chart for carbonyl protein content and age

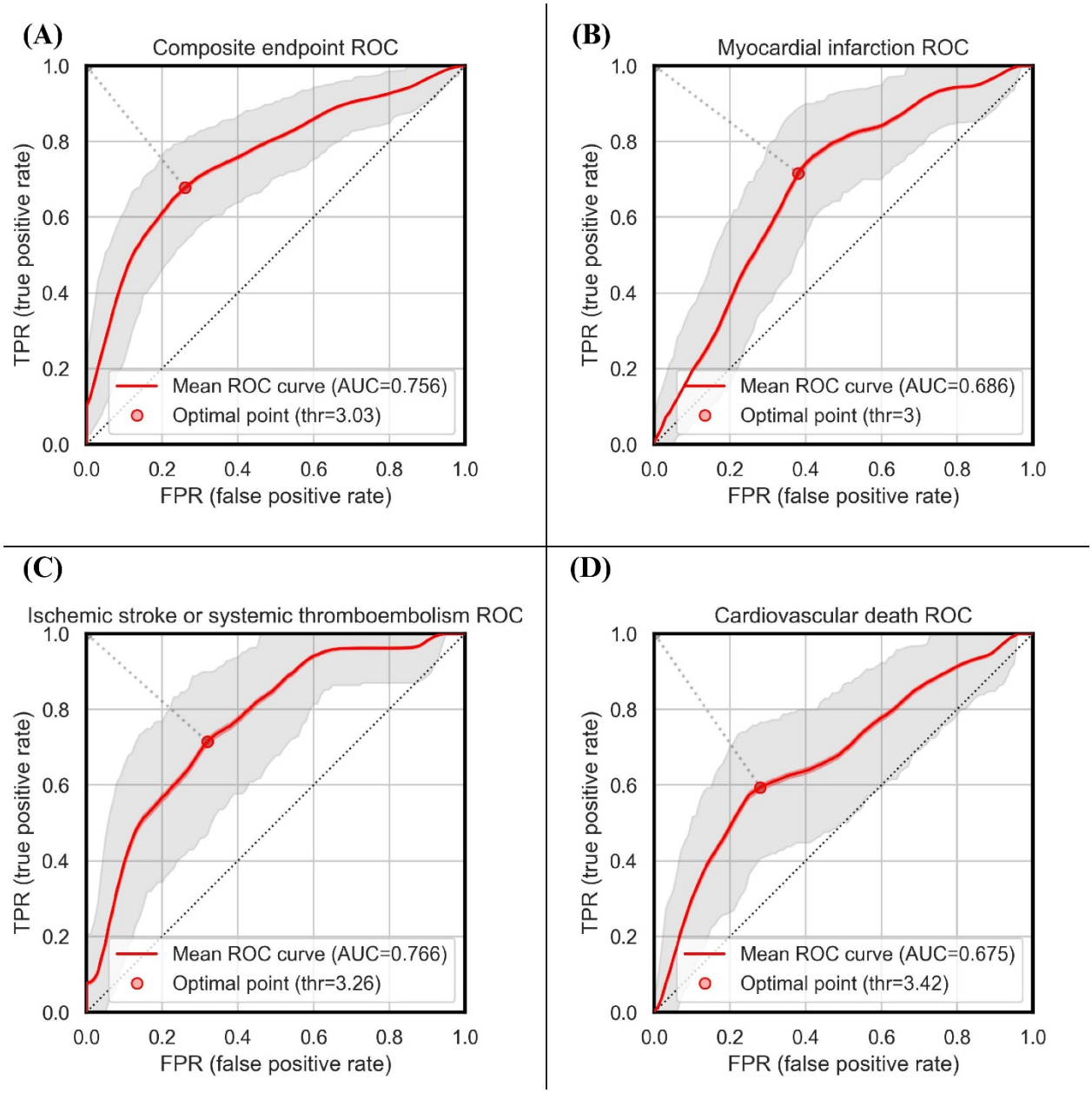

Figure S3. Receiver operating characteristic (ROC) curves for carbonylated protein content (PC) as a predictor of an event occurring during follow-up. Optimal cutoff was selected by optimization of Youden's J statistic (threshold, in nmol/mg, given in each plot)

The optimal cut-off value for baseline PC calculated by optimization of Youden's J statistic was 3.03 nmol/mg protein with an area under the curve (AUC) of 0.753; 95% CI, 0.678–0.827 for the composite endpoint, 3.00 nmol/mg protein for myocardial infarction (AUC 0.685; 95% CI, 0.592–0.778), 3.26 nmol/mg protein for ischemic stroke or systemic thromboembolism (AUC 0.767; 95% CI, 0.671–0.864), and 3.42 nmol/mg protein for cardiovascular death (AUC 0.675; 95% CI, 0.561–0.785)