Received: 16 August 2022 | Accepted: 30 October 2022

DOI: 10.1111/eci.13900

ORIGINAL ARTICLE

WILEY

Serum proprotein convertase subtilisin/Kexin type 9 and vascular disease in type 2 diabetic patients

Giuseppe Armentaro[1] | **Federico Carbone**[2,3] | **Velia Cassano**[1] | **Luca Liberale**[2,3] | **Silvia Minetti**[2] | **Maria Bianca Bertolotto**[2] | **Gaia Mannino**[1] | **Teresa V. Fiorentino**[1] | **Maria Perticone**[1] | **Elena Succurro**[1] | **Giorgio Sesti**[4] | **Fabrizio Montecucco**[2,3] | **Francesco Andreozzi**[1] | **Angela Sciacqua**[1]

[1]Department of Medical and Surgical Sciences, University Magna Græcia of Catanzaro, Catanzaro, Italy

[2]First Clinic of Internal Medicine, Department of Internal Medicine, University of Genoa, Genoa, Italy

[3]IRCCS Ospedale Policlinico San Martino Genoa, Genoa, Italy

[4]Department of Clinical and Molecular Medicine, Sapienza University of Rome, Rome, Italy

Correspondence
Velia Cassano, Department of Medical and Surgical Sciences, University Magna Græcia of Catanzaro, Catanzaro 88100, Italy.
Email: velia.cassano@libero.it

Abstract

Background: Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) levels have been suggested as novel atherosclerotic biomarker. PCSK9 plays important roles in the pathogenesis of atherosclerosis by regulating the degradation of low-density lipoprotein receptor as well as different inflammatory pathways. Considering the important prognostic role of arterial stiffness in cardiovascular disease (CVD), the aim of the study is to investigate the correlation between PCSK9 levels and arterial stiffness in a cohort of diabetic patients, without previous CV events.

Methods: This cross-sectional analysis enrolled 401 Caucasian patients with type II diabetes mellitus (T2DM). PCSK9 levels were measured by ELISA test, arterial stiffness was estimated by measuring carotid-femoral pulse wave velocity (PWV).

Results: Patients were divided in three tertiles according to increasing value of PCSK9. From the I to the III tertiles, there was a significant increase in high sensitivity C-reactive protein (hs-CRP), fibrinogen and white blood cells (WBC) and a reduction in estimated glomerular filtration rate (e-GFR). Patients with higher levels of PCSK9 presented increased systolic, diastolic blood pressure, pulse pressure and PWV. PWV was significantly and directly correlated with PCSK9, fibrinogen, age, BMI and PP, and indirectly correlated with diet, lifestyle and e-GFR. Serum PCSK9 was the major predictor of PWV, justifying a 16.9% of its variation.

Conclusion: Our study demonstrates a close association between circulating PCSK9 levels and PWV in T2DM subjects without previous CV events even after adjusting for well-known CV risk factor and pharmacological medications. Serum PCSK9 could be a useful biomarker for CV risk stratification in diabetic subjects.

KEYWORDS

arterial stiffness, atherosclerosis, hypertension, PCSK9, PWV, type 2 diabetes

Giuseppe Armentaro and Federico Carbone have contributed equally as first authors to this work. Fabrizio Montecucco, Francesco Andreozzi and Angela Sciacqua have contributed equally as last authors to this work.

1 | INTRODUCTION

Proprotein convertase subtilisin/kexin type 9 (PCSK9), a molecule mainly expressed in the liver with detectable circulating levels, is implicated in the degradation of low-density lipoprotein receptor (LDL-R), and, as a consequence in the regulation of LDL plasma circulating levels.[1] *PCSK9* overexpression or gain-of-function associates with increased LDL cholesterol (LDL-C) plasma concentration and cardiovascular (CV) risk.[2] Monoclonal antibodies to PCSK9 have been approved in lowering LDL-C plasma levels, and they have demonstrated to reduce the incidence of CV events.[3,4]

Recent studies suggested that PCSK9 could carry out systemic functions on organs and tissues other than liver regardless of its effect on plasma LDL-C levels.[5] Among them, PCSK9 is directly involved in the progression of atherosclerosis by increasing the expression of pro-inflammatory genes, promoting apoptotic cell death and endothelial dysfunction. Accordingly, PCSK9 was found in the atherosclerotic plaques where it is expressed by vascular smooth muscle cells (VSMC).[5,6]

Arterial stiffness is a widely studied surrogate marker of vascular damage and it is related to increased risk for CV diseases (CVD) and renal dysfunction in different populations.[7] Since plaque formation, arterial stiffening and increased carotid intima media wall thickness (IMT) share similar underlying mechanisms, it is plausible that the presence of PCSK9 in atherosclerotic plaques could play a role also in arterial wall remodelling.[8,9] Accordingly, a study conducted by Cohen and co-workers demonstrated that PCSK9 was associated with modification in carotid IMT in a large population,[10] furthermore data obtained in a Korean cohort of hypertensive subjects, demonstrated that PCSK9 concentration was associated with IMT regardless of CV risk factors.[11] Also, serum levels of PCSK9 were recently reported to predict increased carotid IMT in asymptomatic subjects, regardless of traditional CV risk factors such as hypertension, obesity and LDL-cholesterol.[12] Lastly, in a large sample of overall healthy participants from the Brisighella Heart Study, after adjusting for well-known CV risk factors, circulating PCSK9 levels were significantly associated with pulse wave velocity (PWV), an early marker of arterial stiffness and an independent predictor of CV events.[13]

Emerging data suggest PCSK9 as a risk factor for type 2 diabetes mellitus (T2DM) with several preclinical and clinical studies indicating a role for this molecule in glycometabolic disturbances and as a predictor of CV outcome in diabetic patients with coronary artery disease (CAD).[14–16]

Yet, reports are often conflicting and whether PCSK9 levels could work as a reliable marker of vascular stiffness in such population remains unclear.[17] The aim of the present study is to evaluate the associations between circulating PCSK9 levels and arterial stiffness assessed as carotid-femoral PWV.

2 | DESIGN AND METHODS

2.1 | Study population

We enrolled $n = 401$ T2DM, Caucasian patients (241 males and 160 females, mean age 60 ± 11 years), with a disease duration ≤5 years and under pharmacological treatment, participating to CATanzaro MEtabolic RISk factors study (CATAMERI).[18] All patients underwent physical examination and review of their medical history. All subjects had normal renal function [estimated glomerular filtration rate (e-GFR) > 60 ml/min/1.73 m^2] and postmenopausal women with hormone replacement therapy were not included. No patient had a history or clinical evidence of angina, myocardial infarction, valvular heart disease, familial hypercholesterolemia, peripheral vascular disease, coagulopathy, or any disease predisposing to vasculitis or Raynaud's phenomenon. Other exclusion criteria included history of alcohol and/or drug abuse, as well as secondary forms of hypertension.

The ethics Committee approved the protocol (code protocol number 2012.63) and informed consent was obtained from all participants. All investigations were carried out in accordance with the Declaration of Helsinki.

2.2 | Blood pressure measurements

Readings of clinical bloody pressure (BP) were obtained in the left arm of the supine patients after 5 min of rest, with a sphygmomanometer. Minimum three measurements were taken on three separate occasions, at least 2 weeks apart. Baseline BP values were the average of the last two of the three consecutive measurements obtained at interval of 3 min. Subjects with systolic BP (SBP) >140 mmHg and/or diastolic BP (DBP) >90 mmHg were considered hypertensive.[19]

2.3 | Laboratory determinations

All laboratory measurements were performed after a fasting period of at least 12 h on peripheral blood samples. Circulating levels of PCSK9 were measured by colorimetric ELISA test (R&D Systems, Minneapolis, MN). Analytical determinations were taken using an automatic

particle counter (Siemens Healthcare Diagnostics ADVIA 120/2120 Haematology System) to measure white blood cell count (WBC). T2DM was defined in accordance to the American Diabetes Association (ADA) criteria.[20] Plasma glucose was measured by glucose oxidation method (Beckman Glucose Analyser II; Beckman Instruments), and plasma insulin concentration was determined by a chemiluminescence-based assay (Roche Diagnostics). Glycated haemoglobin (HbA1c) was measured with high-performance liquid chromatography using a National Glicohemoglobin Standardization Program (NGSP) certified automated analyser (Adams HA-8160 HbA1c analyser).

Triglyceride (TG) and total, LDL and HDL cholesterol concentrations were measured by enzymatic methods (Roche Diagnostics). Fibrinogen was determined with automated nephelometric technology using the BNTMII analyser (Siemens Healthcare). Serum levels of high sensitivity C-reactive protein (hs-CRP) were measured by immunoturbidimetric method with an automated system (CardioPhase hsCRP). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured using the a-ketoglutarate reaction (Roche). Creatinine was measured by using Jaffe methodology. Renal function was then quantified by e-GFR according to the equation suggested by the Chronic Kidney Disease Epidemiology Collaborating Group (CKD-EPI).[21]

2.4 | Determination of serum PCSK9 circulating levels

Serum PCSK9 circulating levels were measured by colorimetric enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Minneapolis), according to the manufacturer's instructions. The limit of detection was 0.6 ng/ml. Mean intra and interassay coefficients of variation (CV) were below 8%.

2.5 | Arterial stiffness

Arterial stiffness was estimated by analysing the shape and speed of peripheral ad central pressure waves. All analysis were performed in supine patients, in a quiet room with a constant temperature between 22°C and 24°C, after abstaining from cigarette smoking, food and alcohol intake in the 12 h preceding the study. The measurements were obtained by a validated system (Sphygmocor™; AtCor Medical) that employs high-fidelity applanation tonometry (Millar) and appropriate computer software for the analysis of pressure wave (Sphygmocor™).[7] Pressure calibration was obtained through automatically, noninvasively recorded supine brachial artery BP of the dominant arm after 30 min of rest (Dinamap Compact T; Johnson & Johnson Medical Ltd.). BP was measured five times over 10 min, and the mean of the last three measurements was taken for calibration. Pressure wave recording was performed at the radial artery of the dominant arm with the wrist softly hyperextended, and it is the average of single pressure waves recorded consecutively for 8 s. Pressure wave recordings were accepted only if variation of peak and bottom pressures of single pressure waves was <5%. The central pressure wave was automatically derived from the radial pressures by a built-in generalized transfer function. Moreover, pressure wave measurement was also obtained at the right carotid artery, as it is well known that central Augmentation Index (AI) may be more accurately derived from this vascular site. Central waveforms were further analysed to identify the time to peak/shoulder of the first (T1) and second (T2) pressure wave components during systole. The pressure at the peak/ shoulder of T1 was identified as outgoing pressure wave height (P1), and the pressure at the peak/shoulder of T2 was identified as the reflected pressure wave height (P2), either absolutely or as percent of ejection duration. Augmentation Pressure (AP) was defined as the difference between P2 and P1, and AI as [AP/pulse pressure (PP)] × 100. Aortic pulse wave velocity (PWV) was determined from the carotid and femoral pressure waveforms.

2.6 | Statistical analysis

Baseline characteristics are reported as mean ± SD for continuous variables and frequencies and percentages for categorical data. Normality of distributions of continuous variables was assessed using the Kolmogorov–Smirnov test. Differences in anthropometric, clinical and biological parameters between the three tertiles of increasing PCSK9 values were evaluated by ANOVA for continuous variables and by Bonferroni post hoc test for multiple comparisons between groups. The Chi-square test was used for nominal data. The differences were considered statistically significant for p values < .05. A linear regression analysis was performed on the whole study population with the aim to investigate the possible correlation between PWV, considered as a dependent variable, and the different covariates. Subsequently, variables significantly related to PWV were included in a stepwise multiple regression model to define the strongest determinants of arterial stiffness. The entire statistical analysis was conducted using the SPSS V20.0 program for Windows (SPSS Inc.).

3 | RESULTS

3.1 | Study population

The entire cohort of $n = 401$ patients was divided into tertiles according to increasing values of serum PCSK9 levels. Mean PCSK9 values were 303.6 ± 217.7 ng/ml in the whole population and 88.7 ± 45.6 ng/ml, 262.5 ± 67.8 and 558.1 ± 150.7 in the I, II and III tertile, respectively.

Table 1 reports demographic, anthropometric and biochemical characteristics of the whole study population and subgroups of PCSK9 tertiles. There were no significant differences between the three subgroups in terms of age, smoking, body mass index (BMI), HbA1c, LDL and HDL-cholesterol, TG, AST and ALT. By contrast, the I tertile showed a higher number of female patients in comparison with the other two tertiles. In addition, from I to III tertile, a statistically significant increase was observed for high-sensitivity C reactive protein (hs-CRP) ($p = .032$), WBC ($p = .004$) and fibrinogen levels ($p < .0001$), while e-GFR showed a reduction ($p = .034$).

Bonferroni's post hoc analysis for multiple comparison between groups confirmed that patients with higher PCSK9 values (III tertile) were characterized by elevated hs-CRP values ($p = .04$), higher WBC levels ($p = .004$) and fibrinogen ($p < .0001$) and reduced e-GFR values ($p = .033$) in comparison with I tertile patients. Moreover, fibrinogen ($p = .005$) and WBC ($p = .043$) values were significantly lower in patients from the II tertile when compared with those in the III one.

Table 2 reports comorbidities and pharmacological treatment of the whole study population and according to tertiles of serum PCSK9. Patients in the III tertile showed a greater prevalence of arterial hypertension with no significant difference in the use of the various drug classes. In addition, III tertile presented a significant lower attitude to lifestyle changes and a higher use of lipid-lowering drugs.

Regarding the antidiabetic therapy, there was no significant difference among tertiles for insulin therapy, sulphonylureas and incretins, on the contrary I and III tertiles showed a greater use of biguanides.

TABLE 1 Anthropometric and biochemical characteristics of the study population taken as a whole as well as divided into tertiles according to the increasing levels of circulating PCSK9

	Whole cohort ($n = 401$)	I Tertile ($n = 133$)	II Tertile (n = 134)	III Tertile ($n = 134$)	p**
Gender, males/females	241/160	94/39	74/60	73/61	.009*
Smokers, *n* (%)	70 (17.5)	23 (17.3)	22 (16.4)	25 (18.7)	.888
Age, years	60.0 ± 11.0	59.2 ± 10.5	59.6 ± 11.5	61.1 ± 11.0	.349
PCSK9, ng/ml	303.6 ± 217.7	88.7 ± 45.6	262.5 ± 67.8	558.1 ± 150.7	<.0001
BMI, kg/m^2	31.9 ± 6.5	32.0 ± 5.9	31.5 ± 6.7	32.0 ± 6.8	.775
HbA1c, %	7.3 ± 1.7	7.4 ± 1.7	7.1 ± 1.5	7.5 ± 1.9	.295
LDL, mg/dl	117.7 ± 40.0	114 ± 39.6	120.1 ± 37.9	120.2 ± 42.9	.418
HDL, mg/dl	46.7 ± 14.0	46.2 ± 13.9	48.0 ± 15.0	45.9 ± 12.8	.421
TG, mg/dl	157.2 ± 101.4	149.7 ± 92.9	162.6 ± 127.2	159.4 ± 78.2	.561
AST, UI/L	24.2 ± 14.2	22.5 ± 11.4	24.8 ± 13.6	25.7 ± 17.5	.221
ALT, UI/L	29.9 ± 23.5	27.0 ± 16.9	31.2 ± 25.6	32.0 ± 27.5	.216
e-GFR, ml/min/1,73m^2	108.8 ± 36.2	115.4 ± 36.9	107.4 ± 37.0	103.9 ± 33.9	.034[a]
Fibrinogen, mg/dl	336.3 ± 89.6	307.4 ± 79.5	329.6 ± 83.9	365.3 ± 94.1	<.000[b]
WBC, cells/μl	7484.4 ± 2113.8	7141.4 ± 2100.8	7338.1 ± 1902.3	7971.1 ± 2250.1	.004[c]
hs-CRP, mg/L	5.4 ± 1.4	3.7 ± 1.3	6.0 ± 1.6	6.4 ± 1.8	<.032[d]

Note: Data are expressed as mean ± SD. *χ^2 test. **Differences between groups (ANOVA).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; e-GFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; hs-CRP, highly sensitive C reactive protein; LDL, low-density lipoproteins; TG, triglycerides; WBC, white blood cells.

Bonferroni post hoc test:

[a]Group 1 vs. Group 3, $p = .033$.

[b]Group 1 vs. Group 3, $p < .0001$; Group 2 vs. Group 3, $p = .005$.

[c]Group 1 vs. Group 3, $p = .004$; Group 2 vs. Group 3, $p = .043$.

[d]Group 1 vs. Group 3, $p = .040$.

3.2 | Haemodynamic parameters

Peripheral and central haemodynamic parameters of the whole study population and according to tertiles of PCSK9 are reported in Table 3. Notably, patient with higher PCSK9 values showed significantly higher values of peripheral SBP ($p < .0001$), DBP ($p = .043$), pulse pressure (PP) ($p = .004$) and central PP ($p = .044$). Moreover, also vascular stiffness indices AI ($p = .003$) and PWV ($p < .0001$) were significantly increased in the III tertile. Bonferroni's post hoc analysis showed that patients in the I tertile of PCSK9 values presented significantly lower SBP

TABLE 2 Comorbidities and medication distribution within the study population taken as a whole as well as divided into tertiles according to the increasing levels of circulating PCSK9

	Whole cohort ($n = 401$)	I Tertile ($n = 133$)	II Tertile ($n = 134$)	III Tertile ($n = 134$)	p
Arterial hypertension, *n* (%)	340 (84.8)	103 (77.4)	112 (83.6)	125 (93.3)	.001
Dyslipidaemia, *n* (%)	163 (40.6)	46 (34.6)	63 (47)	54 (40.3)	.117
RAASi, *n* (%)	236 (58.9)	71 (53.4)	77 (57.5)	88 (65.7)	.115
CCBs, *n* (%)	81 (20.2)	20 (15)	28 (20.9)	33 (24.6)	.144
Beta-blockers, *n* (%)	85 (21.2)	23 (17.3)	32 (23.9)	30 (22.4)	.385
Loop Diuretics, *n* (%)	133 (33.2)	35 (26.3)	50 (37.3)	48 (35.8)	.117
Statins, *n* (%)	120 (29.9)	38 (28.6)	29 (21.6)	53 (39.5)	.005
Other lipid-lowering drugs, *n* (%)	20 (5)	6 (4.5)	7 (5.2)	7 (5.2)	.953
Diet and lifestyle modification, *n* (%)	129 (32.2)	24 (18)	61 (45.6)	44 (32.8)	<.0001
Insulin therapy, *n* (%)	62 (15.5)	17 (12.8)	18 (13.4)	27 (20.1)	.182
Biguanides, *n* (%)	153 (38.2)	57 (42.9)	39 (29.1)	57 (42.5)	.030
Sulphonylureas, *n* (%)	53 (13.2)	11 (8.3)	18 (13.4)	24 (17.9)	.066
Incretins, *n* (%)	9 (2.2)	5 (3.8)	3 (2.2)	1 (0.7)	.251

Note: Data are expressed as mean ± SD. *χ^2 test.

Abbreviations: CCBs, calcium channel blockers; RAASi, inhibitors of the renin-angiotensin-aldosterone system.

TABLE 3 Peripheral and central haemodynamic parameters of the study population taken as a whole as well as divided into tertiles according to the increasing levels of circulating PCSK9

	Whole cohort ($n = 401$)	I Tertile ($n = 133$)	II Tertile ($n = 134$)	III Tertile ($n = 134$)	p**
SBP, mmHg	135.8 ± 17,3	134.4 ± 13.6	137 ± 15.6	139.6 ± 18.9	<.0001[a]
DBP, mmHg	79.9 ± 10.3	78.1 ± 10.6	80.8 ± 9.9	80.9 ± 10.3	.043
PP, mmHg	55.9 ± 15	52.6 ± 13.7	56.2 ± 13.5	58.7 ± 16.9	.004[b]
Central-SBP, mmHg	126.9 ± 17.6	125.9 ± 18.8	126.8 ± 13.6	127.8 ± 19.9	.683
Central-DBP, mmHg	84.9 ± 13.4	82.8 ± 11.3	85.4 ± 17.1	86.3 ± 10.5	.086
Central-PP, mmHg	41.9 ± 15.7	40.5 ± 11.9	40.5 ± 16.8	44.7 ± 17.5	.044
AP, mmHg	11.5 ± 7.9	12.3 ± 9.1	10.4 ± 8.1	12.0 ± 6.3	.117
AI, %	26.3 ± 12.4	24.0 ± 12.4	25.9 ± 12.6	29.1 ± 11.8	.003[c]
PWV, m/s	8.3 ± 2.7	7.3 ± 2.1	8.1 ± 2.5	9.4 ± 3.1	<.0001[d]

Note: Data are expressed as mean ± SD. *χ^2 test. **Differences between groups (ANOVA).

Abbreviations: AI, augmentation index; AP, augmentation pressure; DBP, diastolic blood pressure; PP, pulse pressure; PWV, pulse wave velocity; SBP, systolic blood pressure.

Bonferroni post hoc test:

[a]Group 1 vs. Group 2, $p = .008$; Group 1 vs. Group 3, $p < .0001$.

[b]Group 1 vs. Group 3, $p = .003$.

[c]Group 2 vs. Group 3, $p = .002$.

[d]Group 1 vs. Group 2, $p < .0001$; Group 1 vs. Group 3, $p < .0001$.

values in comparison with both II ($p = .008$) and III tertile ($p < .0001$).

In addition, PP ($p = .003$) and AI ($p = .002$) were significantly lower in I tertile compared with III tertile but not significantly different between II and III tertile.

3.3 | Correlation analysis

A linear regression analysis was performed, in the whole study population, to test the correlation between PWV as a dependent variable and different covariates (Table 4).

PWV resulted directly and significantly correlated with PCSK9 circulating levels ($r = .408$, $p = .003$), fibrinogen ($r = .131$, $p < .0001$), age ($r = .322$, $p = .004$), BMI ($r = .308$ $p = .001$) and PP ($r = .125$, $p = .006$) and inversely correlated with diet and lifestyle ($r = -.122$, $p = .024$) and e-GFR ($r = -.107$, $p = .016$). There was no significant correlation between PWV and gender, smoking, hs-CRP, WBC, aortic PP, lipid profile, HbA1c, lipid-lowering drugs, oral antidiabetic drugs, insulin therapy, loop diuretics and the use of other antihypertensive drugs. Variables reaching statistical significance were inserted in a stepwise multivariate linear regression model to determine the independent predictors of PWV. As shown in Table 5, PCSK9 circulating levels resulted in the major predictor of PWV explaining a 16.9% of variation, age, BMI and diet/lifestyle added another 10.6%, 9.5% and 1.6%, respectively. The entire model accounted for a 38.6% of PWV variation.

TABLE 4 Linear regression analysis conducted on PWV, considered as a dependent variable, in the entire study population

	PWV	
	r	*p*
PCSK9, ng/ml	0.408	.003
Age, years	0.322	.004
BMI, kg/m^2	0.308	.001
Fibrinogen, mg/dl	0.131	<.0001
PP, mmHg	0.125	.006
hs-CRP, mg/L	0.076	.999
WBC, U/mm^3	0.066	.094
PPc, mmHg	0.066	.095
Gender, m/f	0.023	.321
LDL, mg/dl	0.017	.412
Triglycerides, mg/dl	0.020	.233
HbA1c	0.003	.122
Diet and lifestyle, *n* (%)	−0.122	.024
eGFR, ml/min/1.73 m^2	−0.107	.016
Oral antidiabetic drugs,	−0.080	.167
RAASi	−0.080	.142
HDL, mg/dl	−0.054	.146
Lipid-lowering drugs, *n* (%)	−0.049	.117
Smoking, mg/dl	−0.046	.087
Insulin therapy, *n* (%)	−0.009	.141
CCBs	−0.003	.264
Loop diuretics	−0.007	.441

Abbreviations: BMI, body mass index; CCBs, calcium channel blockers; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; hs-CRP, highly sensitive C reactive protein; LDL, low-density lipoproteins; PCSK9, Proprotein convertase subtilisin/kexin type 9; PP, pulse pressure; PPc, pulse pressure central; RAASi, inhibitors of the renin angiotensin aldosterone system; WBC, white blood cells.

TABLE 5 Stepwise multiple regression analysis conducted on PWV, considered as a dependent variable, in the entire study population

	Partial r^2	Total r^2	*p*
PCSK9, ng/ml	16.9	16.9	<.0001
Age, years	10.6	27.5	<.0001
BMI, kg/m^2	9.5	37.0	<.0001
Diet, *n* (%)	1.6	38.6	.033

Abbreviations: BMI, body mass index; PCSK9, Proprotein convertase subtilisin/kexin type 9.

4 | DISCUSSION

We demonstrated a direct and independent correlation between serum PCSK9 levels and arterial stiffness assessed by measuring carotid-femoral PWV in a large cohort of 401 patients with T2DM under pharmacological treatment and with no previous CV events.

T2DM patients are characterized by high rate of CV morbidity and mortality,[22] therefore they might profit from an early stratification of the disease burden to improve clinical outcome. Arterial stiffness is a characteristic of T2DM-related vascular diseases and may favour microvascular disease in patients with T2DM, by increasing PWV; microvascular disease in turn contributes to systemic vascular dysfunction by decreasing nitric oxide bioavailability in the blood vessels.[23] PWV represents a largely used parameter for an indirect evaluation of arterial stiffness and it is able to predict CV events in different clinical settings.[24]

Our results demonstrated that in T2DM patients without CV complications, increased serum PCSK9 levels associate with increased values of PWV. Specifically, patients in the III tertile of PCSK9 presented a worse haemodynamic profile, higher values of PWV and its haemodynamic correlates AI and AP. Notably, in the stepwise multivariate linear regression analysis, after adjustment

for several confounding factors including pharmacological treatment, PCSK9 circulating levels represented the strongest determinant of PWV accounting for a 16.9% of its variation. Considering the prognostic role of PWV this issue is clinically relevant.

The peculiarity of our study is that we demonstrated a positive correlation between PCSK9 plasma levels and PWV, as shown by the results of the linear correlation ($r = .408$, $p = .003$), in a cohort of Caucasian T2DM patients. Indeed, previously similar results were reported only by Guo and co-workers in Asiatic individuals.[25] It is important to underline how genetic background may affect clinical results, in fact circulating plasma levels of PCSK9 greatly vary among individuals and may be influenced by genetic factors.[17] Previous reports indicated PCSK9 difference in people from different ethnic groups, with Hispanic people showing higher levels when compared with African-Americans and European-Americans with average levels.[26] Moreover, Lakoski and colleagues examined the consequences of sequence variations in PCSK9 alleles on plasma levels, and demonstrated that African-Americans, who were heterozygous for nonsense mutations in PCSK9 (PCSK9:Y142X or PCSK9:C679X), presented lower median values of PCSK9 than those without a null allele.

Our results are in agreement with the Brisighella Heart study, conducted overall in a healthy population, which demonstrated that circulating levels of PCSK9 correlated with PWV values.[27] A further study conducted by Lee et al showed that patients with high circulating PCSK9 values had increased IMT values, suggesting a possible role of PCSK9 as an early predictor of atherosclerosis, regardless of metabolic factors, including lipid profile.[11] Despite its known roles in LDL-C regulation and therefore in atherosclerosis, recent studied showed that PCSK9 can accelerate plaque growth by mechanism independent of hepatic LDL-R and related to inflammation.[12,28] Accordingly, a cohort study that included over 4000 participants showed that baseline plasma PCSK9 concentrations were correlated to the risk of CVD incidence in the absence of elevated LDL-cholesterol after 15 years of follow-up.[29] Moreover, a recent study conducted by Toscano and collaborators confirmed that PCSK9 is positive correlated to PWV in a cohort of heterozygous familial hypercholesterolemia and demonstrated that treatment with PCSK9-inibhitors was able to significantly reduce PWV other than in LDL-cholesterol.[30]

Of interest, data from our study showed that there was no significant difference in LDL-cholesterol value among tertiles, however patients in the III tertile of PCSK9 had increased levels of both hs-CRP and WBC, indicating the presence of chronic low grade of inflammation.[12]

Another interesting result emerged from our study is that patients with higher serum levels of circulating PCSK9 had increased fibrinogen values, compared with subjects belonging to the I and II tertile. Fibrinogen is considered not only an inflammatory index, but also a new biomarker of atherosclerotic diseases, in fact, in a study conducted by Zhang et al., patients with high levels of PCSK9, presented higher values of fibrinogen; suggesting that the circulating levels of PCSK9 and fibrinogen were positively correlated with atherosclerosis.[31] However, the specific mechanisms underlying the interaction between PCSK9 and fibrinogen require further investigation.

In addition, growing evidence supports a direct role of PCSK9 in the vascular damage. In particular, several studies indicate that PCSK9 could accelerate atherosclerosis by promoting vascular inflammation, which causes endothelial dysfunction, promotes the formation of atherosclerotic plaques and their vulnerability to rupture.[24] The increased expression of PCSK9 determines overexpression of the lectin-like ox-LDL receptor-1 (LOX-1 receptor), which appears to be up-regulated in inflammation and its activation promotes oxidized LDL uptake and the increase in oxidative stress thus amplifying the inflammatory process with negative effects on vascular remodelling.[13] The consideration that PCSK9 is expressed in VSMC as well as in human atherosclerotic plaques may further support this hypothesis.[5,27] Finally, it is known the prognostic role of PWV that has been imputed to negative haemodynamic effects of aortic stiffening with an increase in both aortic SBP and PP with higher left ventricular systolic load and reduced coronary perfusion pressure. Of interest, this study has demonstrated a strong association between PCSK9 circulating levels and arterial stiffness, in diabetic patients, independently of well-known CV risk factors and drug treatment. Moreover, our cohort of T2DM patients presented a well-regulated glycaemic control, with HbA1c average between 7%–7.5%; probably in a cohort with less beneficial HbA1c%, we would have obtained higher plasma PCSK9 values[32] and worse arterial stiffness.

5 | CONCLUSION

Our study demonstrated a close direct and independent association between circulating levels of PCSK9 and PWV in a Caucasian population of T2DM individuals. Herein reported results support the important correlation between PCSK9 and arterial stiffness and the potential role of such mediator in the early identification of T2DM subjects at higher CV risk. In conclusion, it can be inferred that PCSK9 can be considered both a marker of vascular risk and a therapeutic target, as treatment with PCSK9

inhibitors has been shown to reduce PWV in patients with familial hypercholesterolemia.

AUTHOR CONTRIBUTIONS

AS and FM designed the research; VC, GA, FC, LL, SM, MBB and GM performed the research; FC, LL, SM, MBB, GM and TVF collected the data; AS, FM and FA analysed the data; VC and GA wrote the article; FA, GS, ES, TVF, FM and AS revised the manuscript.

ACKNOWLEDGEMENT

None.

CONFLICT OF INTEREST

The Authors declare they have not conflict of interest.

ORCID

Giuseppe Armentaro https://orcid.org/0000-0001-9775-9715
Federico Carbone https://orcid.org/0000-0003-2957-4078
Velia Cassano https://orcid.org/0000-0002-0793-1225
Luca Liberale https://orcid.org/0000-0003-1472-7975
Elena Succurro https://orcid.org/0000-0003-2578-692X
Fabrizio Montecucco https://orcid.org/0000-0003-0823-8729

REFERENCES

1. Cao YX, Jin JL, Sun D, et al. Circulating PCSK9 and cardiovascular events in FH patients with standard lipid-lowering therapy. *J Transl Med*. 2019;17(1):367.
2. Paciullo F, Fallarino F, Bianconi V, Mannarino MR, Sahebkar A, Pirro M. PCSK9 at the crossroad of cholesterol metabolism and immune function during infections. *J Cell Physiol*. 2017;232:2330-2338.
3. Robinson JG, Farnier M, Krempf M, et al. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. *N Engl J Med*. 2015;372(16):1489-1499.
4. Sabatine MS, Giugliano RP, Wiviott SD, et al. Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. *N Engl J Med*. 2015;372(16):1500-1509.
5. Cesaro A, Bianconi V, Gragnano F, et al. Beyond cholesterol metabolism: the pleiotropic effects of proprotein convertase subtilisin/kexin type 9 (PCSK9) genetics, mutations, expression, and perspective for long-term inhibition. *BioFactors*. 2020;46:367-380.
6. Reeskamp LF, Tromp TR, Hovingh GK. PCSK9 as predictor for recurrent cardiovascular disease in familial hypercholesterolemia. *Prev Cardiol*. 2021;28:270-271.
7. Cassano V, Crescibene D, Hribal ML, et al. Uric acid and vascular damage in essential hypertension: role of insulin resistance. *Nutrients*. 2020;12(9):2509.
8. Mattace-Raso FUS, Van Der Cammen TJM, Hofman A, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam study. *Circulation*. 2006;113(5):657-663.
9. Laurent S, Katsahian S, Fassot C, et al. Aortic stiffness is an independent predictor of fatal stroke in essential hypertension. *Stroke*. 2003;34(5):1203-1206.
10. Cohen JC, Boerwinkle E, Mosley TH, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med*. 2006;354(12):1264-1272.
11. Lee CJ, Lee YH, Park SW, et al. Association of serum proprotein convertase subtilisin/kexin type 9 with carotid intima media thickness in hypertensive subjects. *Metabolism*. 2013;62(6):845-850.
12. Li S, Guo YL, Xu RX, et al. Association of plasma PCSK9 levels with white blood cell count and its subsets in patients with stable coronary artery disease. *Atherosclerosis*. 2014;234(2):441-445.
13. Van Bortel LM, Laurent S, Boutouyrie P, et al. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *J Hypertens*. 2012;30:445-448.
14. Peng J, Zhu CG, Li JJ. The predictive utility of circulating PCSK9 levels on diabetes mellitus. *Cardiovasc Diabetol*. 2021;20(1):45.
15. Shi J, Zhang W, Niu Y, et al. Association of circulating proprotein convertase subtilisin/kexin type 9 levels and the risk of incident type 2 diabetes in subjects with prediabetes: a population-based cohort study. *Cardiovasc Diabetol*. 2020;19(1):209.
16. Peng J, Liu MM, Jin JL, et al. Association of circulating PCSK9 concentration with cardiovascular metabolic markers and outcomes in stable coronary artery disease patients with or without diabetes: a prospective, observational cohort study. *Cardiovasc Diabetol*. 2020;19(1):167.
17. Han J, Wang X, Ye P, et al. Plasma PCSK9 levels are unrelated to arterial stiffness in a community-based, 4.8-year prospective study. *J Hum Hypertens*. 2017;31(11):720-724.
18. Andreozzi F, Succurro E, Mancuso MR, et al. Metabolic and cardiovascular risk factors in subjects with impaired fasting glucose: the 100 versus 110 mg/dL threshold. *Diabetes Metab Res Rev*. 2007;23(7):547-550.
19. Williams B, Mancia G, Spiering W, et al. Practice guidelines for the management of arterial hypertension of the European society of cardiology and the European society of hypertension ESC/ESH task force for the management of arterial hypertension. *J Hypertens*. 2018;36:2284-2309.
20. American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes. *Diabetes Care*. 2019;42:S13-S28.
21. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-612.
22. Sharif S, Visseren FLJ, Spiering W, De Jong PA, Bots ML, Westerink J. Arterial stiffness as a risk factor for cardiovascular events and all-cause mortality in people with type 2 diabetes. *Diabet Med*. 2019;36(9):1125-1132.
23. Antonopoulos AS, Siasos G, Oikonomou E, et al. Arterial stiffness and microvascular disease in type 2 diabetes. *Eur J Clin Invest*. 2021;51(2):e13380.
24. Sciacqua A, Maio R, Miceli S, et al. Association between one-hour post-load plasma glucose levels and vascular stiffness in essential hypertension. *PLoS One*. 2012;7(9):e44470.
25. Guo W, Gong Y, Li J, et al. Association of serum proprotein convertase subtilisin/kexin type 9 with early atherosclerosis

in newly diagnosed type 2 diabetes mellitus. *Nutr Metab Cardiovasc Dis*. 2019;29(8):815-821.
26. Lakoski SG, Lagace TA, Cohen JC, Horton JD, Hobbs HH. Genetic and metabolic determinants of plasma PCSK9 levels. *J Clin Endocrinol Metab*. 2009;94(7):2537-2543.
27. Ruscica M, Ferri N, Fogacci F, et al. Circulating levels of proprotein convertase subtilisin/kexin type 9 and arterial stiffness in a large population sample: data from the Brisighella heart study. *J Am Heart Assoc*. 2017;6(5):e005764.
28. Ortega E, Gilabert R, Nuñez I, et al. White blood cell count is associated with carotid and femoral atherosclerosis. *Atherosclerosis*. 2012;221(1):275-281.
29. Leander K, Mälarstig A, Van'T Hooft FM, et al. Circulating proprotein convertase subtilisin/kexin type 9 (PCSK9) predicts future risk of cardiovascular events independently of established risk factors. *Circulation*. 2016;133(13):1230-1239.
30. Toscano A, Cinquegrani M, Scuruchi M, et al. PCSK9 plasma levels are associated with mechanical vascular impairment in familial hypercholesterolemia subjects without a history of atherosclerotic cardiovascular disease: results of six-month add-on PCSK9 inhibitor therapy. *Biomolecules*. 2022;12(4):562.
31. Zhang Y, Zhu CG, Xu RX, et al. Relation of circulating PCSK9 concentration to fibrinogen in patients with stable coronary artery disease. *J Clin Lipidol*. 2014;8(5):494-500.
32. Momtazi AA, Banach M, Pirro M, Stein EA, Sahebkar A. PCSK9 and diabetes: is there a link? *Drug Discov Today*. 2017;22:883-895.

How to cite this article: Armentaro G, Carbone F, Cassano V, et al. Serum proprotein convertase subtilisin/Kexin type 9 and vascular disease in type 2 diabetic patients. *Eur J Clin Invest*. 2023;53:e13900. doi: 10.1111/eci.13900