Association of proprotein convertase subtilisin/kexin type 9 (PCSK9) levels with abnormally high ankle-brachial index in atrial fibrillation

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ABSTRACT

Background: High ankle-brachial index (ABI) has been associated with increased risk of worse outcomes in the general population. Few data on atrial fibrillation (AF) exist. Experimental data suggest that proprotein convertase subtilisin/kexin type 9 (PCSK9) contributes to vascular calcification but clinical data on this association are lacking.

Aims: We wanted to investigate the relationship between circulating PCSK9 levels and an abnormally high ABI in patients suffering from AF.

Methods: We analyzed data from 579 patients included in the prospective ATHERO-AF study. An ABI≥1.4 was considered high. PCSK9 levels were measured coincidentally with ABI measurement. We used optimized cut-offs of PCSK9 for both ABI and mortality obtained from Receiver Operator Characteristic (ROC) curve analysis. All-cause mortality according to the ABI value was also analyzed.

Results: One hundred and fifteen patients (19.9%) had an ABI ≥1.4. The mean (standard deviation [SD]) age was 72.1 (7.6) years, and 42.1% of patients were women. Patients with ABI ≥1.4 were older, more frequently male, and diabetic. Multivariable logistic regression analysis showed an association between ABI ≥1.4 and serum levels of PCSK9 >1150 pg/ml (odds ratio [OR], 1.649; 95% confidence interval [CI], 1.047–2.598; $P = 0.031$). During a median follow-up of 41 months, 113 deaths occurred. In multivariable Cox regression analysis, an ABI ≥1.4 (hazard ratio [HR], 1.626; 95% CI, 1.024–2.582; $P = 0.039$), CHA_2DS_2-VASc score (HR, 1.249; 95% CI, 1.088–1.434; $P = 0.002$), antiplatelet drug use (HR, 1.775; 95% CI, 1.153–2.733; $P = 0.009$), and PCSK9 >2060 pg/ml (HR, 2.200; 95% CI, 1.437–3.369; $P < 0.001$) were associated with all-cause death.

Conclusions: In AF patients, PCSK9 levels relate to an abnormally high ABI ≥1.4. Our data suggest PCSK9 role in contributing to vascular calcification in AF patients.

Key words: ABI, atrial fibrillation, mortality, PCSK9, peripheral artery disease

INTRODUCTION

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is an enzyme involved in cholesterol metabolism inducing intracellular degradation of hepatic low-density lipoprotein cholesterol (LDL-C) receptors, reducing serum LDL-C [1].

In addition to its cholesterol-related action, PCSK9 may represent a risk factor for cardiovascular disease patients through several mechanisms, including reactive oxygen species generation [2], platelet activation [3], and direct modifications of blood vessels walls [4], contributing to an accelerated vascular calcification [4–6].

Increased PCSK9 has been found in some cardio-metabolic diseases such as hyperten-

WHAT'S NEW?

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is associated with an abnormally high ankle-brachial index (ABI) ≥1.4 in anticoagulated atrial fibrillation patients. High serum PCSK9 and ABI ≥1.4 are associated with increased mortality risk in this patient population.

sion and diabetes mellitus and is associated with increased risk of cardiovascular events (CVEs) in some cardiovascular settings, including atrial fibrillation (AF) [3].

AF patients are, in particular, characterized by an atherosclerotic burden, with a high prevalence and incidence of coronary artery disease and peripheral arterial disease (PAD) [7].

The prevalence of PAD is difficult to estimate, given the high proportion of undiagnosed patients. Currently, it is estimated that a population of >200 million patients worldwide may suffer from PAD [8]. PAD carries an increased risk of CVEs and major adverse limb events including vascular re-intervention and deaths [9–11], leading to a reduction in quality of life and life expectancy. For this, reason, the European Society of Cardiology (ESC) Guidelines [12] recommend screening patients at risk of PAD using the ankle-brachial index (ABI), which represents an evidence-based, useful, and low-cost screening tool for asymptomatic PAD. In addition to its diagnostic value, the ABI has also prognostic implications, as either low (≤0.9) or high (>1.3) ABI has been associated with increased risk of CVEs and cardiovascular death [13].

Indeed, also in the AF population, an earlier prospective study showed an increased risk of CVEs in patients with an impaired ABI <0.9 compared to a normal ABI (0.9–1.3) [14]. However, few data on clinical characteristics of AF patients with high ABI >1.3 have been reported so far. Based on this, we investigated the relationship between circulating PCSK9 levels and an abnormally high ABI in patients suffering from AF enrolled in the prospective ATHERO-AF study.

METHODS

We analyzed data on consecutive patients with non-valvular AF enrolled in the prospective observational ATHERO-AF at the Department of Clinical, Internal, Anaesthesiologic and Cardiovascular Sciences, Sapienza University of Rome. The ATHERO-AF study started in February 2008 and is still ongoing (ClinicalTrials.gov Identifier: NCT01882114). In 2013, the study protocol was amended to include a second arm of patients treated with direct oral anticoagulants. The current analysis was performed on the cohort of patients on vitamin K antagonists (VKAs). Patients were regularly monitored every 2–3 weeks according to international normalised ratio (INR) values. The follow-up of each patient was stopped when one of the primary endpoints occured (as previously defined in [15]).

The quality of anticoagulation was calculated by the time in therapeutic range (TiTR) as previously described [16]. During the first clinical examination, a complete personal medical history was collected, including drug therapy and comorbidities.

Cardiovascular risk factors

Definitions of cardiovascular risk factors such as hypertension, diabetes mellitus, and heart failure have been previously reported according to international guidelines [17–20].

Optimized risk factors were defined according to the Atrial fibrillation Better Care (ABC) pathway, as previously described [21]. Thus, well-controlled hypertension was defined as blood pressure <140/90 mm Hg, and treatment with angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin receptor blockers (ARB) was considered a standard of care for the management of heart failure [21]. Optimized treatment for previous myocardial infarction/coronary revascularization was considered the use of beta-blockers [21].

Inclusion criteria

All patients presenting with non-valvular AF who were >18 years of age were eligible for the study. All patients were treated with VKAs after appropriate thrombotic risk stratification [22] (international normalized ratio target: 2.5).

Exclusion criteria

Exclusion criteria were prosthetic heart valves, severe cognitive impairment, chronic infections (human immunodeficiency virus infection, hepatitis C virus, hepatitis B virus), or systemic autoimmune disease. Patients with an ABI <0.9 or overt PAD were excluded.

Lipid profile and PCSK9 serum levels assessment

Patients were also asked to collect a blood sample. At baseline, a lipid profile was obtained, including total cholesterol, high-density lipoprotein cholesterol (HDL-C) (mg/dl), and triglycerides (mg/dl). Low-density lipoprotein cholesterol (LDL-C, mg/dl) was calculated by the validated Friedewald formula. Also, creatinine (mg/dl) with glomerular filtration rate (GFR) (ml/min/1.73 m^2) by the simplified Modification of Diet in Renal Disease (MDRD) formula, and fasting blood glucose (mg/dl) were obtained.

The obtained blood samples were taken into tubes with 3.8% sodium citrate and centrifuged at 300 g for 10 min to obtain supernatant, then immediately stored at –80°C until use. Plasma levels of PCSK9 were measured by a commercial enzyme-linked immunoadsorbent assay (Boster Biological Technology 3942 B Valley Ave, Pleasanton, CA

94566) [3]. Plasma samples were diluted 1:10 in a diluent buffer. Data were expressed as pg/ml, and the minimal detectable dose of PCSK9 was <10 pg/ml in human plasma. The assay and interassay coefficients of variance were 5.8% and 6.9%, respectively.

Ankle-brachial index measurement

The ABI was calculated as the ratio of systolic blood pressure obtained from the ankle and brachial arteries in all AF patients using an 8 MHz CW Vascular Doppler (Risingmed Model: RFD-B). All procedures followed guidelines and recommendations [23]. Ankle and brachial systolic blood pressures were measured both on the right and left side, and the ABI was assessed separately for the right and left leg using the highest arm and ankle pressures. A value of ABI ≥1.4 was considered abnormally high. Patients with an ABI <0.9 were excluded, and those with an ABI 0.9–1.3 were used as a reference group.

Statistical analysis

Categorical variables were reported as numbers and percentages which were compared with Pearson's χ^2 test. Mean and standard deviation (SD) or median and interquartile range (IQR) were used for continuous variables, which were compared by Student's t-test or Mann-Whitney U test, respectively. Normal distribution of variables was checked by the Kolmogorov-Smirnov test. We divided the cohort into two groups according to the ABI values (0.9–1.3 and ≥1.4) to compare baseline clinical characteristics. Stepwise logistic regression analysis was used to calculate the odds ratio (OR) and 95% confidence interval (95% CI) to test the association between clinical factors and high ABI.

All available variables with complete data were included: sex, age ≥75 years, type of AF, diabetes, smoking, heart failure (HF), previous stroke/transient ischemic attack (TIA), previous myocardial infarction/coronary revascularization, antiplatelet, ACE inhibitors/sartans, beta-blockers, digoxin, proton pump inhibitors (PPI), amiodarone, verapamil, and body mass index (BMI). For this model, antihypertensive drugs were entered instead of hypertension, and statins instead of cholesterol levels. For the analysis, we used an optimized cut-off of PCSK9 >1150 pg/ml obtained from diagnostic Receiver Operator Characteristic (ROC) curve analysis (sensitivity 60%, specificity 48%).

Cox proportional hazards regression analysis was used to calculate the adjusted hazard ratio (HR) and 95%CI of all-cause mortality by each clinical variable. For the survival model, we used the composite CHA_2DS_2-VASc score instead of single variables given the relatively low number of cases. Variables were entered with one step in the multivariable model. Furthermore, we used an optimized cut-off of PCSK9 >2060 pg/ml obtained from ROC curve analysis for mortality (sensitivity 33%, specificity 84%).

All tests were 2-tailed and only *P*-values <0.05 were considered statistically significant. The analyses were performed using SPSS 25.0 software (IBM, Armonk, NY, US).

Sample size calculation

For this post-hoc analysis, we assumed a sampling ratio of 5 to 1 (20% patients with ABI ≥1.4), an HR of 2, and an overall 20% event rate, a sample size of 510 patients guaranteed 80% power for a 5% level test. This number was increased to 579 to guarantee sufficient power and in anticipation of patient loss of 10% during follow-up.

Ethical committee

All patients signed informed written consent at study entry. The study was approved by the local ethic committee of Sapienza University (No. 1306/2007) and was conducted according to the Declaration of Helsinki. The study is registered at clinicaltrials.gov NCT01882114.

RESULTS

The study enrolled 579 patients with AF, of whom 115 (19.9%) had an ABI ≥1.4. The mean (SD) age was 72.1 (7.6) years, and 42.1% of patients were women (Table 1).

Patients with an ABI ≥1.4 were older than patients with ABI 0.9–1.3, and more frequently they were men and were affected by diabetes. Furthermore, patients with ABI ≥1.4 had worse control of blood pressure and had a higher thromboembolic risk (Table 1). There was no difference between ABI groups concerning smoking habits, previous cerebrovascular and cardiovascular diseases, and concomitant therapies (Table 1).

PCSK9 and ABI

In a stepwise multivariable logistic regression analysis, we found an inverse association of female sex and a direct association for diabetes with ABI ≥1.4 (Table 2). We found a significant association between PCSK9 levels >1150 pg/ml and ABI ≥1.4, with an OR 1.649; 95% CI, 1.047–2.598; $P = 0.031$.

ABI, PCSK9, and all-cause mortality

During a median (IQR) follow-up of 41 (23.1–66.1) months, 10 patients in the ABI 0.9–1.3 group were lost and 113 deaths were registered, 27 in the high-ABI group, and 86 in the normal-ABI group (n = 454). In multivariable Cox regression analysis (Table 3), ABI ≥1.4 was associated with increased risk of all-cause of death (hazard ratio [HR], 1.626; 95% CI, 1.024–2.582; $P = 0.039$). Figure 1 shows the adjusted survival curves according to ABI values from multivariable Cox regression analysis. Other predictors of death were CHA_2DS_2-VASc score (HR, 1.249; 95% CI, 1.088–1.434; $P = 0.002$), antiplatelet drug use (HR, 1.775; 95% CI, 1.153–2.733; $P = 0.009$), PCSK9 >2060 pg/ml (HR, 2.200; 95% CI, 1.437–3.369; $P < 0.001$).

DISCUSSION

In this prospective study, we found a direct association between PCSK9 levels and abnormally high values of ABI ≥1.4, which was associated with increased risk of death. We found that nearly 20% of patients showed an abnormally

Table 1. Baseline characteristics of patients with atrial fibrillation according to ankle-brachial index (ABI) values

	Total cohort (n = 579)	ABI 0.9–1.3 (n = 464)	ABI ≥1.4 (n = 115)	*P*-value
Female sex, n (%)	244 (42.1)	213 (45.9)	31 (27.0)	<0.001
Age, years, mean (SD)	72.1 (7.6)	71.6 (7.5)	74.8 (7.4)	0.024
Age ≥75 years, n (%)	233 (40.2)	184 (39.7)	49 (42.6)	0.563
BMI, kg/m^2, mean (SD)	27.5 (4.7)	27.4 (4.6)	27.9 (4.9)	0.339
Persistent/permanent AF, n (%)	287 (49.6)	223 (48.1)	64 (55.7)	0.145
Well-controlled arterial hypertension, n (%)	134 (23.1)	118 (25.4)	16 (13.9)	0.009
T2DM, n (%)	104 (18.0)	67 (14.4)	37 (32.2)	<0.001
Active smoking, n (%)	57 (9.8)	48 (10.3)	9 (7.8)	0.439
Previous stroke/TIA, n (%)	58 (10.0)	48 (10.3)	10 (8.7)	0.593
Previous myocardial infarction/ coronary revascularization, n (%)	126 (21.8)	103 (22.2)	23 (20.0)	0.705
Previous myocardial infarction/ coronary revascularization on beta blockers, n (%)	61 (10.5)	53 (11.4)	8 (7.0)	0.163
Heart failure, n (%)	83 (14.3)	64 (13.8)	19 (16.5)	0.459
Heart failure on ACE-I, n (%)	25 (4.3)	18 (3.9)	7 (6.1)	0.297
CHA_2DS_2-VASc score, mean (SD)	3.2 (1.4)	3.1 (1.3)	3.7 (1.5)	0.021
TiTR %[a], mean (SD)	64.4 (19.8)	65.0 (19.9)	62.1 (19.5)	0.210
PCSK9, pg/ml, median (IQR)	1200 (800–1850)	1200 (800–1900)	1200 (900–2000)	0.219
Fasting blood glucose, mg/dl, mean (SD)	104.0 (28.6)	101.6 (25.9)	113.9 (36.1)	<0.001
Creatinine, mgdl[b], mean (SD)	1.02 (0.33)	1.01 (0.34)	1.04 (0.28)	0.433
eGFR (sMDRD), ml/min/1.73 cm^2[a], mean (SD)	75.1 (22.6)	74.9 (22.6)	75.8 (22.8)	0.741
HDL-C, mg/dl, mean (SD)	47.6 (13.2)	48.1 (13.1)	45.3 (13.3)	0.052
Triglycerides, mg/dl, median (IQR)	103 (86–135)	101.5 (86–133)	110 (82–134)	0.794
LDL-C, mg/dl, mean (SD)	107.6 (30.4)	108.6 (30.0)	103.5 (31.9)	0.123
Therapy				
Antiplatelets, n (%)	104 (18.0)	84 (18.1)	20 (17.4)	0.859
Proton pump inhibitor, n (%)	290 (50.8)	223 (48.7)	67 (59.3)	0.046
Statins, n (%)	268 (46.2)	213 (45.9)	55 (47.8)	0.726
Amiodarone, n (%)	148 (25.6)	121 (26.1)	27 (23.5)	0.633
Digoxin, n (%)	89 (15.4)	72 (15.5)	17 (14.8)	1.000
Verapamil, n (%)	72 (12.4)	58 (12.5)	14 (12.2)	0.924
ACE-I/ARB, n (%)	406 (70.1)	318 (68.5)	88 (76.5)	0.111
Beta-blockers, n (%)	253 (43.7)	204 (44.0)	49 (42.6)	0.834
Oral antidiabetic drugs, n (%)	81 (14.0)	51 (11.0)	30 (26.1)	<0.001
Insulin, n (%)	19 (3.3)	12 (2.6)	7 (6.1)	0.076

[a]Data on 506 patients. [b]Data on 552 patients.

Abbreviations: AF, atrial fibrillation; ACE-I, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; BMI, body mass index; PCSK9, proprotein convertase subtilisin/kexin type 9; T2DM, type 2 diabetes mellitus; TIA, transient ischemic attack; TiTR, time in therapeutic range

Table 2. Stepwise logistic regression analysis of factors associated with ankle-brachial index (ABI) ≥1.4

	Odds ratio	95% confidence interval	*P*-value
PCSK9 >1150 pg/ml	1.649	(1.047–2.598)	0.031
Female sex	0.409	(0.250–0.669)	<0.001
T2DM	2.768	(1.667–4.597)	<0.001
Proton pump inhibitors	1.778	(1.075–2.943)	0.025

See methods for the list of covariates

Abbreviations: see Table 1

Table 3. Multivariable Cox regression analysis model of factors associated with all-cause mortality

	Hazard ratio	95% CI	*P*-value
ABI ≥1.4	1.626	(1.024–2.582)	0.039
Active smoking	1.429	(0.723–2.825)	0.304
Antiplatelet	1.775	(1.153–2.733)	0.009
ACE inhibitors/sartans	0.755	(0.483–1.180)	0.218
Beta-blockers	0.869	(0.561–1.347)	0.531
Digoxin	0.999	(0.598–1.670)	0.998
Verapamil	0.949	(0.543–1.658)	0.853
Amiodarone	0.989	(0.612–1.600)	0.965
Statin	0.800	(0.530–1.207)	0.288
CHA_2DS_2-VASc score	1.249	(1.088–1.434)	0.002
PPI	1.291	(0.858–1.943)	0.221
Persistent/permanent AF	1.401	(0.919–2.135)	0.117
PCSK9 >2060 pg/ml	2.200	(1.437–3.369)	<0.001

Abbreviations: ABI, ankle-brachial index; AF, atrial fibrillation; PPI, proton pump inhibitors; PCSK9, proprotein convertase subtilisin/kexin type 9

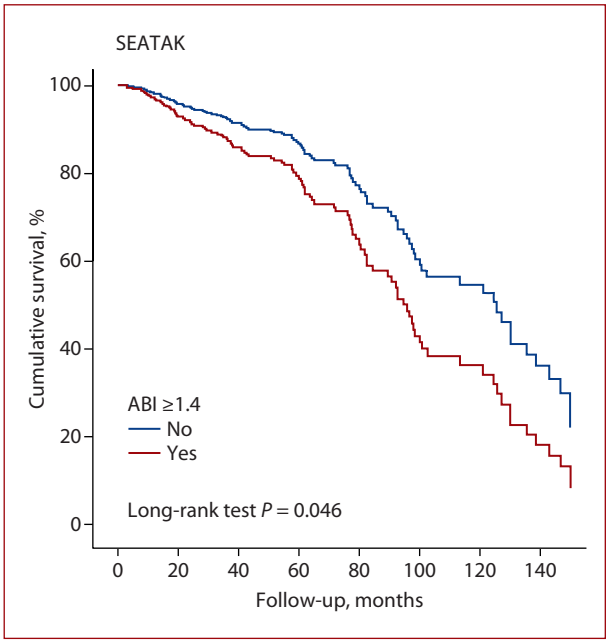

Figure 1. Survival curve from multivariable Cox proportional hazard regression analysis showing a direct association between an ankle-brachial index (ABI) ≥1.4 and all-cause mortality

high ABI ≥1.4. Patients with high ABI were more frequently diabetic and less likely to be women. Well-controlled blood pressure was inversely associated with high ABI. This figure is slightly lower than in a previously reported study that included 287 consecutive anticoagulated AF outpatients, of whom 78 (27%) had an abnormal ABI. In this study, abnormal ABI values were associated with diabetes, heart failure, and ischemic heart disease [24]. Similar findings were reported in a prospective study including 5679 subjects, showing that age, female sex, and diabetes were associated with high ABI [25].

In addition to these already known factors, we also found that the use of proton pump inhibitors (PPI) was associated with vascular calcification. This finding is in keeping with previous evidence showing that the use of PPI is associated with vascular calcification of aortic and iliac arteries [26, 27] and increased risk of calcinosis [28]. The mechanism for this association is not completely elucidated but experimental evidence suggested that the use of PPI may influence metalloproteinase (MMP) activity. In particular, omeprazole induced vascular MMP-2 expression and activity, resulting in increased media thickness and vascular oxidative stress [29].

A novel finding of our study is the significant association between PCSK9 levels and a high ABI. A previous experimental study showed that human and rat smooth muscle cells (SMCs), overexpressing PCSK9 showed a higher extracellular calcium deposition compared to control SMCs when exposed to a pro-calcific environment [30]. Furthermore, the same cells showed an increase in pro-calcific markers, such as bone morphogenetic protein 2 and alkaline phosphatase, and a concomitant decrease in anti-calcific mediator matrix GLA protein and osteopontin [30]. In addition, in asymptomatic patients with familial hypercholesterolemia, PCSK9 levels were increased in patients with coronary artery calcification as compared to those without [31].

Our findings add to these results providing clinical evidence that PCSK9 may contribute in vivo to vascular calcification also in patients with cardiovascular disease, such as those with non-valvular AF. Patients with AF on treatment with VKAs represent a high-risk group of patients in terms of vascular complications, as VKA therapy itself represents a risk factor for vascular calcification. Hence, previous evidence showed that VKAs were associated with human vascular smooth muscle cell calcification and atherosclerotic plaque progression and increased coronary artery calcification [32–34]. Indeed, the use of VKAs may aggravate vascular calcification in patients with an already abnormally high ABI, especially when elevated PCSK9 levels coexist.

We also analyzed the relationship between high ABI and incidence of mortality and found an increased risk of death in AF patients with high ABI. Our results are in keeping with the study by Velescu et al. [25], in which an ABI ≥1.4 was independently associated with all-cause mortality (HR, 2.0; 95% CI, 1.32–2.92) in subjects without cardiovascular disease at baseline [25]. The previously mentioned study by Gallego et al. also showed a similar association with all-cause mortality (adjusted HR, 2.76; 95% CI, 1.08–7.06; $P = 0.033$) in AF patients [24].

Among predictors of mortality, we found that concomitant antiplatelet drug administration is associated with increased mortality rate. This evidence is in line with previous studies showing that mortality may be the result of increased bleeding events [35–37].

In terms of clinical implications of the study, further studies are needed to investigate if patients with a high ABI may benefit from switching to a direct oral anticoagulant to reduce the risk of vascular calcification progression, especially if a high PCSK9 concentration is present. In this context, the value of PCSK9 in refining clinical risk in AF patients compared to other biomarkers may be studied [38]. In this regard, the variability of PCSK9 levels over time needs further investigation, as some factors such as high-dose statins may increase PCSK9 levels [39].

Limitations

Our study included elderly Caucasian patients with a relatively low proportion of patients with diabetes. Thus, our results should be confirmed in studies with other ethnic groups and with a higher percentage of metabolic patients. A second limitation was that patients were anticoagulated with VKAs, which did not allow us to observe if a similar result could be obtained in subjects treated with direct oral anticoagulants. Given the observational design of the study, we could only deduce association but not causality between PCSK9 levels and ABI values. Furthermore,

despite the analysis being adjusted for the most common cardiovascular risk factors, residual confounding was likely to remain. Finally, we used a commercial assay to measure PCSK9 levels, but it could not discriminate among various forms of circulating PCSK9.

In conclusion, patients with AF and ABI ≥1.4 have a higher risk of cardiovascular events and demonstrate high PCSK9 levels. The role of PCSK9 in contributing to vascular calcification in vivo needs further investigation.

Article information

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