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Article Number: 884378

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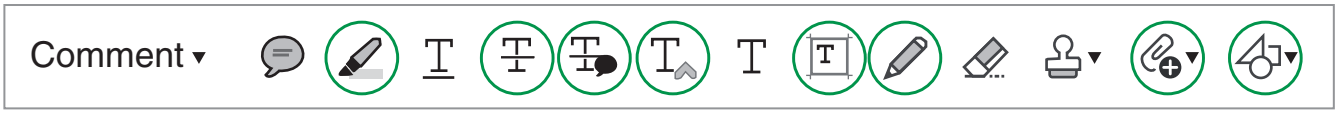

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European Journal of Preventive Cardiology

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European Journal of Preventive Cardiology
0(00) 1–4

DOI: 10.1177/2047487319884378
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Angiopoietin-like 3 and subclinical peripheral arterial disease: Evidence from the Brisighella Heart Study

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Angiopoietin-like 3 (ANGPTL3) is a 70 kDa protein primarily expressed in the liver. The main physiological role is related to the inhibition of lipoprotein and endothelial lipases, thus affecting triglyceride hydrolysis.[1] The role of ANGPTL3 in atherosclerosis has emerged from genetics: carriers of loss of function (LOF) mutations of ANGPTL3, leading to low plasma levels, had a reduced risk of coronary artery disease and a lipid profile characterised by dramatic reductions of low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides.[2] However, although homozygous or compound heterozygous for ANGPTL3 LOF had a reduced capacity to promote cell cholesterol efflux, no clinical evidence of accelerated atherosclerosis was found.[3] ANGPTL3 also influences the endothelial cell adhesion and stimulates the proliferation of haematopoietic stem cells,[4] both potentially atherosclerosis associated.

As, to date, in a general population no data have been reported on a possible direct involvement of ANGPTL3 with arterial disease, the present retrospective study was aimed to evaluate the relationship between ANGPTL3 plasma levels and extra-coronary arterial health, as assessed by the ankle–brachial blood pressure index (ABI), a validated, non-invasive tool for peripheral artery disease (PAD) screening.[5] Moreover, levels of proprotein convertase subtilisn/kexin type 9 (PCSK9), which may play a crucial role in vascular aging, were also evaluated.[6]

The Brisighella Heart Study is a longitudinal population study on a randomly allocated sample representative of the entire population of Brisighella, a rural northern Italian village. The study, carried out in agreement with the Declaration of Helsinki, was approved by the institutional ethics board of the University Hospital of Bologna.[7]

Plasma ANGPTL3 and PCSK9 were determined by commercial enzyme-linked immunosorbent assay (ELISA) kits.[8] Blood pressure was measured according to the guidelines of the European Society of Cardiology and of the European Society of Hypertension. ABI, augmentation index (AI) and carotid-femoral pulse wave velocity (cfPWV) were measured by a Vicorder apparatus (Skidmore Medical Ltd., UK).[9] Patients with no history of clinical PAD but with a low ABI ($\leq$0.90) were considered as presenting with subclinical PAD.

A bivariate correlation between age, blood pressure, heart rate, LDL-cholesterol, HDL-cholesterol, triglycerides, lipoprotein (a) (Lp(a)), serum uric acid (SUA), estimated glomerular filtration rate (eGFR), cfPWV and ABI was investigated. In a logistic regression analysis, ABI was considered as a dependent variable, whereas gender, physical activity, cfPWV, Lp(a), PCSK9, ANGPTL3, SUA and eGFR were considered as independent variables. Data were age and LDL-cholesterol adjusted.

Among 2939 subjects, after a screening on PAD based on the ABI measurement, 1968 subjects who participated in the 2016 survey were selected. These comprise a sample of 241 post-menopausal women (52.5%) and 218 age-matched men (47.5%) with normal ($<$1.10 ABI $<$1.40) or abnormal ($\leq$0.90 or $\geq$1.40) ABI values. Treatment with statins or vasodilating agents was

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considered as exclusion criteria as was the case for borderline ABI values (between 0.91 and 1.10).[10] Menopausal age was based on patient self-reporting of menstrual cessation.

As reported in Table 1, subjects with optimal ($n = 378$) or abnormal ($n = 81$) ABI values were age-matched (67.6 vs. 68.1 years), with no differences in haemodynamic characteristics, i.e. blood pressure, AI and cfPVW. Thirty-six per cent of subjects were hypertensive and 33% were overweight. When the lipid profile was evaluated, no statistically significant differences were found between the two groups. No-one had a diagnosis of diabetes. Conversely, differences between groups were found for baseline eGFR, significantly lower in subjects with a more compromised ABI, i.e. 59.4 ± 8.9 versus 66.6 ± 9.0 ($P < 0.001$) and for PCSK9 levels, significantly lower in less compromised subjects, i.e. 336.1 ± 69.3 ng/mL versus 354.2 ± 67.9 ng/mL ($P = 0.036$). In the entire cohort, PCSK9 levels were normally distributed, with a mean value of 342.4 ± 69.1 ng/mL. ANGPTL3 was not normally distributed (133.2 ± 50.8 ng/mL) and consequently was log-transformed before further analyses.

Multivariate analyses adjusted for age and LDL-cholesterol and carried out on the whole population showed that ABI was mainly predicted by log ANGPTL3 (odds ratio (OR) 1.110, 95% confidence interval (CI) 1.008–1.192, $P < 0.001$), PCSK9 (OR 1.127, 95% CI 1.063–1.221, $P = 0.001$) and SUA (OR 1.203, 95% CI 1.104–1.311, $P < 0.001$). This last, along with chronic kidney failure, are considered as emerging risk factors for PAD. Gender, physical activity, Lp(a), eGFR and cfPWV were not associated with ABI.

The significant predictors of ABI in the different subgroups are reported in Table 2. In both men and women, the parameter best associated with ABI was SUA; only in men ABI was further predicted by ANGPTL3 ($\beta = 1.099$, 95% CI 1.042–1.219) and PCSK9 ($\beta = 1.129$, 95% CI 1.007–1.268) levels. When subjects were classified based on normal or abnormal ABI values, ANGPTL3 was an ABI predictor in both groups ($\beta = 1.103$, 95% CI 1.018–1.134 and $\beta = 1.184$, 95% CI 1.018–1.223, respectively), whereas SUA and PCSK9 levels were predictive parameters only in the abnormal ABI group. When subjects were classified as having normal or impaired renal function, ANGPTL3 and PCSK9 remained significant ABI predictors in both groups, whereas SUA was predictive only in subjects with preserved renal function.

In the general Caucasian population PAD is the third leading cause of atherosclerotic cardiovascular morbidity, after coronary artery disease and stroke,

Table 1. Main characteristics of the selected subjects.

	Normal ABI ($n = 378$)		Abnormal ABI ($n = 81$)		
Parameters	Mean	SD	Mean	SD	*P* value
Age (years)	67.6	4.6	68.1	5.1	0.095
BMI (kg/m^2)	27.1	3.8	27.1	4.1	0.978
SBP (mmHg)	136.6	12.8	139.5	19.6	0.566
DBP (mmHg)	75.2	8.4	73.3	7.7	0.086
AI	28.2	7.3	29.4	8.1	0.242
cfPWV (m/s)	9.5	2.3	9.9	2.7	0.290
ABI	1.1	0.1	0.8	0.1	<0.001
Total cholesterol (mg/dL)	227.1	29.1	216.9	32.9	0.054
LDL-cholesterol (mg/dL)	149.7	26.6	141.3	31.9	0.074
HDL-cholesterol (mg/dL)	51.6	5.2	51.0	6.1	0.756
Triglycerides (mg/dL)	129.9	49.5	122.4	41.8	0.376
Lipoprotein (a) (mg/dL)	24.8	11.5	22.0	9.1	0.469
PCSK9 (ng/mL)	336.1	69.3	354.2	67.9	0.036
Angiopoietin-like 3 (ng/mL)	132.9	52.5	134.5	41.9	0.806
Fasting plasma glucose (mg/dL)	96.0	7.4	97.1	8.3	0.592
Serum uric acid (mg/dL)	5.3	1.2	5.2	1.2	0.357
eGFR (ml/min)	66.6	9.0	59.4	8.9	<0.001

ABI: ankle brachial index; AI: augumentation index; BMI: body mass index; cfPWV: carotid–femoral pulse wave velocity; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; HDL: high-density lipoprotein; LDL: low-density lipoprotein; PCSK9: proprotein convertase subtilisin/kexin type 9; SBP: systolic blood pressure; SD: standard deviation.

Table 2. Predictors of ABI in prespecified subgroups.

Subgroups		Predictor	β	95% CI		*P* value
				Lower	Upper	
Sex	Men	SUA	1.143	1.031	1.199	<0.001
		PCSK9	1.129	1.007	1.268	0.002
		ANGPTL-3	1.099	1.042	1.219	0.013
	Women	SUA	1.319	1.208	1.376	0.005
Ankle brachial index	Normal ABI	ANGPTL-3	1.103	1.018	1.134	<0.001
	Abnormal ABI	SUA	1.201	1.112	1.359	<0.001
		PCSK9	1.123	1.012	1.255	0.009
		ANGPTL-3	1.184	1.018	1.223	0.031
Renal function	eGFR ≥90 ml/min	SUA	1.234	1.138	1.341	<0.001
		PCSK9	1.119	1.037	1.239	0.010
		ANGPTL-3	1.099	1.008	1.167	<0.001
	eGFR <90 ml/min	PCSK9	1.193	1.035	1.323	0.011
		ANGPTL-3	1.091	1.001	1.188	0.048

ABI: ankle brachial index; ANGPTL-3: angiopoietin-like 3; CI: confidence interval; eGFR: estimated glomerular filtration rate; PCSK9: proprotein convertase subtilisin/kexin type 9; SUA: serum uric acid.

with a prevalence of approximately 3.5%. In order to prevent the frequently rapid disease progression, early identification of PAD and its risk factors are strongly suggested.[11,12] ABI measurement is considered as most appropriate for PAD screening in the general population. Low ABI (<0.9) values are associated with a significant risk of all-cause (risk ratio (RR) 2.52, 95% CI 2.26–2.82) and cardiovascular mortality (RR: 2.94, 95% CI 2.72–3.18) as well as of cerebrovascular events (RR 2.17, 95% CI 1.90–2.47).[13] In the described cohort, ANGPTL3 was found to be significantly associated with suboptimal ABI in overall healthy elderly people.

In healthy volunteers, ANGPTL3 was positively associated with femoral artery intima media thickness independent of age, sex, smoking, body mass index, lipids, systolic blood pressure and the insulin resistance index.[14] Thus, in humans, ANGPTL3 may be closely associated with arterial wall thickness both in the carotid and femoral beds.

While ANGPTL3 was associated with ABI in subjects with both normal and abnormal ABI, when PCSK9 was considered the association was present only in the subgroup with abnormal ABI. In the same population, we have previously demonstrated that PCSK9 was directly correlated to arterial stiffness, as assessed by cfPWV.[15] It is thus reasonable to conclude that ANGPTL3 could be an early predictor of PAD, whereas elevated PCSK9 may be a reliable biomarker of the disease severity.

The possible mechanism of the association between ANGPTL3 and abnormal ABI may be ascribed to a direct vascular wall damage exerted by ANGPTL3, independent of triglyceride metabolism, and explained by the following evidence: (a) the C-terminal fibrinogen-like domain of ANGPTL3 can bind to the integrin $\alpha_V\beta_3$ expressed on endothelial cells; (b) the binding of ANGPTL3 to integrin $\alpha_V\beta_3$ may facilitate the development of atherosclerotic plaques by promoting intraplaque angiogenesis; (c) integrin $\alpha_V\beta_3$ is involved in foam cell formation and inflammation.[16] In conclusion, in a large group of healthy mature subjects, ABI was significantly associated with ANGPTL3 levels, particularly in women.

Acknowledgements

The authors would like to acknowledge the Faenza Public Health District and all the General Practitioners of Brisighella for their continuous support of the study.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: this work was supported by the University of Bologna and the Fondazione del Monte (Bank Foundation), Cariplo Foundation (2015-0552) and intramural grant Università degli Studi di Milano (PSR 2018) to MR.

References

1. Lu X. Structure and function of angiopoietin-like protein 3 (ANGPTL3) in atherosclerosis. *Curr Med Chem*. Epub

ahead of print 22 June 2019. DOI: 10.2174/0929867326666190621120523.
2. Stitziel NO, Khera AV, Wang X, et al. ANGPTL3 deficiency and protection against coronary artery disease. *J Am Coll Cardiol* 2017; 69: 2054–2063.
3. Pisciotta L, Favari E, Magnolo L, et al. Characterization of three kindreds with familial combined hypolipidemia caused by loss-of-function mutations of ANGPTL3. *Circ Cardiovasc Genet* 2012; 5: 42–50.
4. Zhang CC, Kaba M, Ge G, et al. Angiopoietin-like proteins stimulate ex vivo expansion of hematopoietic stem cells. *Nat Med* 2006; 12: 240–245.
5. Rossello X, Dorresteijn JA, Janssen A, et al. Risk prediction tools in cardiovascular disease prevention: a report from the ESC Prevention of CVD Programme led by the European Association of Preventive Cardiology (EAPC) in collaboration with the Acute Cardiovascular Care Association (ACCA) and the Association of Cardiovascular Nursing and Allied Professions (ACNAP). *Eur J Prev Cardiol* 2019; 26: 1534–1544.
6. Macchi C, Banach M, Corsini A, et al. Changes in circulating pro-protein convertase subtilisin/kexin type 9 levels – experimental and clinical approaches with lipid-lowering agents. *Eur J Prev Cardiol* 2019; 26: 930–949.
7. Cicero AF, Rosticci M, Bove M, et al. Serum uric acid change and modification of blood pressure and fasting plasma glucose in an overall healthy population sample: data from the Brisighella Heart Study. *Ann Med* 2017; 49: 275–282.
8. Macchi C, Ferri N, Favero C, et al. Long-term exposure to air pollution raises circulating levels of proprotein convertase subtilisin/kexin type 9 in obese individuals. *Eur J Prev Cardiol* 2019; 26: 578–588.
9. 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.
10. Aboyans V, Criqui MH, Abraham P, et al. Measurement and interpretation of the ankle–brachial index: a scientific statement from the American Heart Association. *Circulation* 2012; 126: 2890–2909.
11. Sigvant B, Lundin F and Wahlberg E. The risk of disease progression in peripheral arterial disease is higher than expected: a meta-analysis of mortality and disease progression in peripheral arterial disease. *Eur J Vasc Endovasc Surg* 2016; 51: 395–403.
12. Biondi-Zoccai G, Frati G, Giordano A, et al. Predicting incident peripheral artery disease and critical limb ischemia: feeling the pulse! *Eur J Prev Cardiol*. Epub ahead of print 5 September 2019. DOI: 10.1177/2047487319872018.
13. Hajibandeh S, Hajibandeh S, Shah S, et al. Prognostic significance of ankle brachial pressure index: a systematic review and meta-analysis. *Vascular* 2017; 25: 208–224.
14. Hatsuda S, Shoji T, Shinohara K, et al. Association between plasma angiopoietin-like protein 3 and arterial wall thickness in healthy subjects. *J Vasc Res* 2007; 44: 61–66.
15. 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: ▮▮ [AQ1].
16. Lupo MG and Ferri N. Angiopoietin-like 3 (ANGPTL3) and atherosclerosis: lipid and non-lipid related effects. *J Cardiovasc Dev Dis* 2018; 5: ▮▮ [AQ2].

Appendix 1

The Brisighella Heart Study Group: Arrigo FG Cicero, Elisa Grandi, Federica Fogacci, Marina Giovannini, Elisabetta Rizzoli, Pierangelo Coppola, Fulvio Ventura, Federica Mariasole Piani, Mario Soldati, Ilaria Ricci Iamino, Silvia Palmisano, Matteo Landolfo, Sergio D'Addato, Giuseppe Derosa, Stefano Bacchelli and Claudio Borghi.