sciendo
Internal Medicine 2022 vol. XIX No. 2 - www.srmi.ro
10.2478/inmed-2022-0205

PREDICTORS OF PERIPHERAL ARTERY DISEASE PROGRESSION. IS THERE ANY ROLE FOR VASCULAR AGE?

Raluca Popa[1], Cristina Stănescu[2], Paul Bălănescu[2], Vasile Manoliu[3], Adriana Gurghean[4], Adriana Iliesiu[5]
[1]Dr. Professor Dimitrie Gerota Emergency Hospital
[2]Colentina Clinical Hospital
[3]Politehnica University of Bucharest
[4]Colțea Clinical Hospital
[5]Hospital Professor Dr.Theodor Burghele

Abstract

Background*. Peripheral artery disease (PAD) is a distinct atherosclerotic syndrome marked by stenosis or occlusion of the arteries, particularly of the lower extremities. The major risk factors for PAD are similar to those for coronary and cerebrovascular disease, comprising smoking, hypertension, hypercholesterolemia and diabetes. The factors involved in the progression of PAD are less well defined. Vascular age (VA) is represented by the apparent age of the vascular system derived from the associated cardiovascular risk factors. This concept has been used so far mainly in primary prevention, being usefull for communication with the patient.*

Aim. *The purpose of the study was to investigate the factors involved in the progression of arterial stenosis in patients already having PAD. Moreover, we tried to determine the utility of VA in increasing patient compliance with therapeutic decisions.*

Methods. *Between 1st February 2015 - 31st December 2015, 270 consecutive patients (pts) referred for vascular echo-Doppler assessment were enrolled in the study. 106 pts with non-significant arterial stenosis were prospectively evaluated. All pts underwent complete clinical examination, carotid, femoral ultrasound, echocardiography and laboratory evaluation. Presence of atherosclerotic plaques in the carotid arteries were recorded. Medical history, drug use, smoking behavior, blood pressure, duration of diabetes, presence of premature atherosclerotic PAD in relatives, characterized by disease diagnosis before the age of 50 years, were evaluated. Serum total cholesterol (TC), HDL cholesterol (HDL-C), serum glucose, A1c hemoglobine (HbA1c), serum creatinine, estimated glomerular filtration rate (eGFR) were determined. VA was estimated using Framingham score and intima-media thickness (IMT). The primary end-point was the occurrence of significant peripheral artery stenosis (PAS), defined as a two-fold increase Doppler velocity across the stenosis.*



Internal Medicine

Original Papers

Results. *At baseline, median age of enrolled pts was 57 years, with male predominance (69,8%). Less than 40% had high blood pressure (39.3%), 36.4% had treated hypertension.*

The median left ventricular mass index (LVMI) was 108.5 g/m^2 and concentric hypertrophy was present in 29.9% of patients. Patients with hypertension had LVMI of 113.12 g/m^2 and those without hypertensiun, had 105.6 g/m^2. Diabetes mellitus (DM) was present in 20.6% of patients, majority treated (88%). Among them, 10.3% had more than 10 years duration of DM. Median HbA1c was 5.8%. The majority of the patients are smokers (75.5%). Mean pack-years of smoking was 29.8. During the 4.5 years follow-up, 31.25% quit smoking. The median ejection fraction (EF) was 51.6%.

The median TC concentration was 191.6 mg/dl and median HDL-c level was 62.9 mg/dl. 45.8% of the patients received treatment with statins. Premature atherosclerotic PAD in relatives was present in 25.2 % of the patients, and carotid atheromas (CA) in 35.5%. Aortic calcifications (AC) were found in 37.4% of the patients. Median arterial elastance (Ea) was 2.07 mmHg/ml. Median serum creatinine was 1.03 mg/dl and eGFR was 75.6 ml/ml/1.73 m^2. Median value of the VA calculated by IMT (VA-IMT) was 65.5 years and the median VA calculated by cardiovascular risk factors (VA-RF) was 62.9 years. After 4.5 years of follow-up, 35 (32.7%) patients developed significant PAS. 22 patients had popliteal artey stenosis (62.85%) and 13 patients had superficial femoral artery stenosis (37.15%). During the 4.5 years follow-up, 25 patients (31.25%) quit smoking. Median age of population who developed significant PAS at follow-up was 62.6 years and 39.2% were males.

Individuals who developed stenosis tended to be smokers, older, receiving hypertensive and statin therapy, having diabetes, especially more than 10 years duration, having dyslipidemias and CA. They all had higher VA-IMT and VA-RF values compared to patients that did not develop PAS. Median value of VA-IMT was 72.6 years and VA-RF was 74.2 years ($p<0,001$ for both parameters). They also had significantly higher values of Ea, with median value of 2.16 mmHg/ml. There were no statistically significant differences between the two groups in cardiac performance, LV mass, percentage of LV concentric hypertrophy, presence of AC, premature PAD in relatives, renal function and blood pressure values. In logistic regression analysis, pack-years of smoking, HbA1c %, presence of CA and HDL-C were independently associated with significant PAS progression. From the 25 patients which quit smoking, only 6 developed significant PAS (24%), as compared to 29 (52.7%) from the 55 patients which remained active smokers ($p =0.016$).

Conclusion. *We demonstrated that some of the classical cardiovascular risk factors were involved in PAD progression: smoking, dyslipidemia, diabetes, carotid atherosclerosis. At multivariate analysis the independent variables associated with PAD progression were pack-years of smoking, glycosilated hemoglobin and HDL-cholesterol values and presence of CA. VA assessed by cardiovascular risk scales and by directly measured IMT both predict the progression of PAD, as did Ea, but without independent predictive value for the outcome. Nevertheless, VA was usefull in comunicating with the patients, influencing their perception of disease progression and adherence to therapy, improving shared decision making, mainly quitting smoking. We have shown that even after 4.5 years of smoking cessation there was a measurable effect on PAD progression.*
Keywords: *vascular age, cardiovascular risk factors, peripheral artery disease, intima-media thickness.*

Rezumat

Introducere. *Boala arterială periferică este un sindrom aterosclerotic distinct marcat de stenoză sau ocluzie a arterelor, în special la nivelul extremității inferioare. Factorii majori de risc pentru boala arterială periferică sunt similari cu cei pentru bolile coronariene și cerebrovasculare, incluzând fumatul, hipertensiunea, hipercolesterolemia și diabetul. Factorii implicați în progresia bolii arteriale periferice sunt mai puțin bine definiți. Vârsta vasculară este reprezentată de vârsta aparentă a sistemului vascular, derivată din factorii de risc cardiovascular asociați. Acest concept a fost folosit până acum în principal în prevenția primară, fiind util în comunicarea cu pacientul.*

Scop. *Scopul studiului a fost de a investiga factorii implicați în progresia stenozei arteriale la pacienții care au deja boală arterială periferică. Mai mult, am încercat să determinăm utilitatea vârstei arteriale în creșterea complianței pacientului la deciziile terapeutice.*

Metodă. *În perioada 1 februarie 2015 - 31 decembrie 2015, au fost înrolați în studiu 270 de pacienți consecutivi trimiși pentru evaluarea eco-Doppler vasculară. Au fost evaluați prospectiv 106 pacienți cu stenoză arterială nesemnificativă. Toți pacienții au fost supuși examenului clinic complet, ecografiei carotidiene, femurale, ecocardiografiei și analizelor de laborator. S-a înregistrat prezența plăcilor aterosclerotice în arterele carotide. Au fost evaluate istoricul medical, administrarea de medicamente, fumatul, tensiunea arterială, durata diabetului zaharat, prezența bolii arteriale periferice aterosclerotice premature la rude, caracterizată prin diagnosticarea bolii înainte de vârsta de 50 de ani. S-au determinat colesterolul seric total, colesterolul HDL, glucoza serică, hemoglobina glicozilată, creatinina serică, rata de filtrare glomerulară estimată. Vârsta arterială a fost estimată folosind scorul Framingham și grosimea intimă-medie (IMT). Obiectivul principal a fost apariția stenozei arteriale periferice semnificative, definită ca o creștere de două ori a vitezei Doppler în stenoză.*

Rezultate. *La momentul inițial, vârsta medie a pacienților înscriși a fost de 57 de ani, cu predominanță masculină (69,8%). Mai puțin de 40% aveau hipertensiune arterială (39,3%), 36,4% primeau tratament antihipertensiv. Indicele de masă al ventriculului stâng mediu a fost de 108,5 g/m^2, hipertrofia concentrică fiind prezentă la 29,9% dintre pacienți. Pacienții cu hipertensiune arterială au avut indicele de masă al ventriculului stâng mediu de 113,12 g/m^2, iar cei fără hipertensiune, 105,6 g/m^2.*



Internal Medicine

Original Papers

Diabetul zaharat a fost prezent la 20,6% dintre pacienți, majoritatea tratați (88%). Dintre aceștia, 10,3% au avut diabet zaharat cu o durată mai mare de 10 ani. Media hemoglobinei glicozilate a fost 5,8%. Majoritatea pacienților erau fumători (75,5%). Numărul mediu de pachete-ani fumate a fost de 29,8. Pe parcursul celor 4,5 ani de urmărire, 31,25% s-au lăsat de fumat. Fracția de ejecție medie a fost de 51,6%. Concentrația medie al colesterolului total a fost 191,6 mg/dl și nivelul mediu al colesterolului HDL a fost 62,9 mg/dl, 45,8% dintre pacienți primind tratament cu statine. Boala arterială periferică aterosclerotică prematură la rude a fost prezentă la 25,2% dintre pacienți, iar ateroamele carotidiene la 35,5%. Calcificările aortice au fost găsite la 37,4% dintre pacienți.

Elastanța arterială medie a fost de 2,07 mmHg/ml. Creatinina serică medie a fost de 1,03 mg/dl, iar rata de filtrare glomerulară estimată a fost de 75,6 ml/ml/1,73 m^2. Valoarea medie a vârstei arteriale calculată prin grosimea intimă medie a fost de 65,5 ani, iar vârsta arterială medie calculată prin factorii de risc cardiovascular a fost de 62,9 ani. După 4,5 ani de urmărire, 35 dintre pacienți (32,7%) au dezvoltat stenoză arterială periferică semnificativă. 22 de pacienți au avut stenoză de artere poplitee (62,85%) și 13 pacienți au avut stenoză de arteră femurală superficială (37,15%). Pe parcursul celor 4,5 ani de urmărire, 25 de pacienți (31,25%) au renunțat la fumat. Vârsta medie a populației care a dezvoltat stenoză arterială periferică semnificativă la urmărire a fost de 62,6 ani și 39,2% erau bărbați.

Persoanele care au dezvoltat stenoză au avut tendința de a fi fumători, mai în vârstă, în terapie antihipertensivă și cu statine, având diabet zaharat, în special cu o durată mai mare de 10 ani, având dislipidemii și ateroame carotidiene. Toți au avut valori mai mari ale vârstei arteriale calculată prin grosimea intimă medie și vârstei arteriale calculată prin factorii de risc cardiovascular în comparație cu pacienții care nu au dezvoltat boală arterială periferică. Valoarea medie a vârstei arteriale calculată prin grosimea intimă medie a fost de 72,6 ani, iar a vârstei arteriale calculată prin factorii de risc cardiovascular a fost de 74,2 ani ($p<0,001$ pentru ambii parametri).

Au avut, de asemenea, valori semnificativ mai mari ale elastanței arteriale, cu o valoare medie de 2,16 mmHg/ml. Nu au existat diferențe semnificative statistic între cele două grupuri în ceea ce privește performanța cardiacă, masa ventriculului stâng, procentul de hipertrofie concentrică ventriculară stângă, prezența calcificărilor aortice, boala arterială periferică prematură la rude, funcția renală și valorile tensiunii arteriale. În analiza de regresie logistică, numărul de pachete - ani de fumat, hemoglobina glicozilată, prezența ateroamelor carotidiene și colesterolul HDL au fost asociate în mod independent cu o progresie semnificativă a bolii

arteriale periferice. Dintre cei 25 de pacienți care au renunțat la fumat, doar 6 au dezvoltat boală arterială periferică semnificativă (24%), comparativ cu 29 (52,7%) dintre cei 55 de pacienți care au rămas fumători activi (p =0,016).

Concluzie. *Am demonstrat că unii dintre factorii clasici de risc cardiovascular au fost implicați în progresia bolii arteriale periferice: fumatul, dislipidemia, diabetul, ateroscleroza carotidiană. La analiza multivariată, variabilele independente asociate cu progresia bolii arteriale periferice au fost pachetele - ani de fumat, valorile hemoglobinei glicozilate și HDL-colesterolului și prezența ateroamelor carotidiene. Vârsta arterială evaluată prin scale de risc cardiovascular și prin grosimea intimă-medie măsurată direct prezice progresia bolii arteriale periferice, la fel ca elastanța arterială, dar fără valoare predictivă independentă pentru rezultat. Cu toate acestea, vârsta arterială a fost utilă în comunicarea cu pacienții, influențând percepția acestora asupra progresiei bolii și aderarea la terapie, îmbunătățind procesul decizional comun, în principal prin renunțarea la fumat. Am arătat că chiar și după 4,5 ani de renunțare la fumat a existat un efect măsurabil asupra progresiei bolii arteriale periferice.*

Cuvinte cheie: *vârstă vasculară, factori de risc cardiovascular, boală arterială periferică, grosime intimă-medie.*

Introduction

Peripheral artery disease (PAD) is a distinct atherosclerotic syndrome marked by stenosis or occlusion of the arteries, particularly of the lower extremities. It is estimated that >200 million people have PAD worldwide, with a spectrum of symptoms from none to severe[1].

PAD is strongly associated with concomitant coronary and cerebrovascular diseases. After adjustment for known cardiovascular disease risk factors, PAD is associated with an increased risk of incident coronary and cerebrovascular disease morbidity and mortality[2].

The major risk factors for PAD are similar to those for coronary and cerebrovascular disease. The combination of four clinical risk factors - smoking, hypertension, hypercholesterolemia and diabetes - was strongly and independently associated with risk of confirmed and clinically significant PAD[3, 4].

The factors involved in the progression of PAD are less well defined. Nicoloff et al found that in 5 years, 37% of PAD patients experienced a significant (≥0.15) worsening of ABI (ankle-brachial index), whereas 22% of patients experienced clinical progression of PAD based on a change in symptoms or a need for surgical intervention[5].

In a study of patients with PAD referred to a vascular laboratory, 30.2% of limbs progressed to a more serious category of PAD over an average follow-up time of 4.6 years[6].

Data on risk factors associated with progression of PAD are relatively sparse. In the Cardiovascular Health Study, significant independent predictors of progression were age, cigarette smoking, diabetes mellitus, and dyslipidemia[7].

Smoking is recognized as one of the strongest risk factors for PAD. According to recent data on incident cases of clinical PAD in United States male professionals, smoking is the most contributive risk factor for occurrence of PAD, with a population attributable fraction of



44%[4]. Cessation of smoking among patients with intermittent claudication has been shown to improve various functional and physiological parameters related to PAD, as well as reducing cardiovascular mortality[8].

A lower risk for PAD, coronary artery disease and stroke was observed even within 5 years of smoking cessation[9].

Smoking cessation is known to be very difficult to accomplish. Nowadays, preventive medicine should be based on a patient centred approach, where the therapeutic decisions are taken in collaboration with the patient, who is empowerd to take part in the management of his health state. Explaining to the patient the abstract concept of cardiovascular risk is sometimes difficult. However, the new idea of vascular age (VA) as the age of his arteries is something more easily understood by all patients, improving patient compliance with therapeutic decisions.

VA is represented by the apparent age of the vascular system derived from the associated cardiovascular risk factors. The concept was first brought to atention by Thomas Sydenham, a great English seventeenth-century physician, who said that "A man is as old as his arteries".

VA could be assessed by various instruments like SCORE - mortality or Framingham -morbi-mortality risk scores. VA is determined as the age a person would be with the same calculated cardiovascular risk, but without risk factors[10]. This new concept was incorporated for the first time in cardiovascular risk tables in 2008, as a result of the Framingham study[11]. VA can also be computed from the two SCORE project scales (for high- and low-risk European countries). Agreement of VA calculated from the two SCORE scales was extremely high, in contrast to the poor agreement in absolute risk[10].

Risk scores have been found to overestimate or underestimate the cardiovascular risk. On the other hand, cardiovascular diseases could appear in certain clusters at risk not directly addressed in the conventional risk algorithms. It has been suggested that the value of traditional risk factors such as hypertension, diabetes, dyslipidemia, and smoking for the identification of atherosclerotic burden in an asymptomatic population are limited[12]. As such, estimation of VA by risk scores could have similar limitations. Therefore, estimating VA by other more direct methods could be of added value.

VA represents vascular impairement, which in certain cases could occur prematurely. Early vascular aging (EVA) has been recognized as simultaneously early sign of arteriosclerosis (in the media) and early changes in the endothelial function (in the intima)[13]. As VA is directly linked to the impairment of the arteries, several methods of evaluation emerged, such as the direct evaluation of

common carotid intima-media thickness (IMT) and of arterial stiffnes (AaS).

Ultarsound measurement of IMT, firstly described in 1986[14], has proved to be an important tool for direct assessment of the arterial wall. It is a noninvasive, sensitive, and reproducible technique for quantifying atherosclerotic burden. It was demonstrated that it correlates well with major cardiovascular risk factors and with severity of atherosclerosis in other vascular beds. The predictive value for cardiovascular event, the ability to change over time and to be affected by interventions makes it a suitable surrogate marker for atherosclerosis and cardiovascular disease endpoints[15].

It was shown that IMT could predict the occurence of stroke even in the absence of carotid plaques[16] and across a wide age range (even in people younger than 50 years)[17].

In spite of these consideration, a meta-analysis of prospective studies showed that addition of IMT to the Framingham Risk Score led only to a small improvement in the 10-year risk prediction of first-time myocardial infarction or stroke[18]. Such an improvement is unlikely to be of clinical importance. However, other researchers found considerable evidence that IMT added predictive value primarily in asymptomatic individuals at intermediate cardiovascular risk[19].

A more standardised and accurate measurement of IMT might prove to be valuable for comparing healthy subjects to those with EVA. It is demonstrated that IMT increases with advancing age, as a result of thickening of both the intimal and medial layers. In human beings, IMT increases nearly 3-fold between the ages of 20 and 90 years[20].

In fact, it was the extent of intervention effects on IMT progression that predicted the degree of CVD risk reduction[21]. Based on the reference intervals for common carotid IMT measured with echotracking and on the rate of its progression, a vascular age calculator was developed[22].

AaS reflects the degenerative changes of extracellular matrix in the media layer. It is being characterized by elastin fatigue fracture and collagen deposition and cross-linking. Some experimental studies have demonstrated that changes in extracellular matrix proteins and in the mechanical properties of the vessel wall may activate some pathophysiologic mechanisms involved in the atherosclerotic process. Clinical studies have demonstrated an independent association between AaS and atherosclerotic load[23].

Nevertheless, AaS is distinct from atherosclerosis, a process that typically involves the intima layer, characterized by lipid accumulation, inflammatory cells and vascular smooth muscle cell migration and foam cell development. On the other hand, both processes often coexist in the same vascular territories, share some mutual risk factors, and are part of the vascular aging process[24].

AaS is evaluated usually by means of pulse wave velocity (PWV), estimating the propagation speed of the arterial pulse. It is calculated as a ratio of the distance between two artery recording points, devided by the time requiered for the pressure wave to travel this distance[25].

An other method of evaluating arterial stiffnes is by measuring effective arterial elastance (Ea). Ea, introduced as a measure of the arterial load on the heart[26], is considered also a measure of arterial stiffness[27]. It is approximated by the ratio of left ventricular (LV) end-systolic pressure and stroke volume and combines steady and pulsatile load components[26]. We chose this



method as we considered it more precise, as it takes into account blood pressure.

The purpose of the study was to investigate the factors involved in the progression of arterial stenosis in patients already having PAD. Moreover, we tried to determine the utility of VA in increasing patient compliance with therapeutic decisions.

Method

Study population

Between 1st February 2015-31st December 2015, 270 consecutive patients (pts) referred for vascular echo-Doppler assessment were enrolled in the study. All pts had atherosclerotic plaques in lower limb arteries and 164 had significant arterial stenosis, defined as more than two fold increase of Doppler systolic velocity across the stenosis. Patients with significant peripheral arterial stenosis were excluded from the study. In the end, 106 patients (57.1 ± 10.8 years old, 74 male), with non-significant peripheral arterial stenosis were further evaluated, forming the study group. The study protocol was approved by the Local Ethic Committee and all subjects gave their written informed consent.

Clinical data

All patients underwent complete clinical examination, carotid, femoral ultrasound, echocardiography and laboratory evaluation. Information on cardiovascular risk factors was collected. Presence of *atherosclerotic* plaques in the carotid arteries were recorded. Medical history, drug use, smoking behavior, blood pressure, duration of diabetes, presence of premature atherosclerotic PAD in relatives, characterized by disease diagnosis before the age of 50 years, were evaluated. Also, serum total cholesterol (TC), HDL cholesterol (HDL-C), serum glucose, A1c hemoglobine (HbA1c), serum creatinine, estimated glomerular filtration rate (eGFR) were determined.

Intima-media thickness assessment

The carotid artery was interrogated using an Hitachi Aloka F 37 ultrasound system with linear array transducer of 7,5 MHz in B-mode imaging.

The carotid intima-media thickness (IMT), representing the combined thickness of intima and media layers, was assessed by measuring the combined thickness of the two echogenic lines produced by ultrasound imaging of the far wall of the common carotid artery - the interface lumen-intima and the interface media - adventitia[28]. The measurement was performed in the distal common carotid artery (CCA), at least 5 mm below the distal end of CCA, on a 10-mm-in-length straight arterial segment. We avoided to perform the measurement where a focal lesion was present[29].

Variable	Value (n=106)
Gender (n,%)	Male (69.8%)
Age (years)	57 (34-88)
Smoking (n,%)	80 (75.5%)
Pack-years of smoking (mean ± SD)	29.8 ± 16.1
Diabetes (n,%)	22 (20.6%)
Diabetes more than 10 years duration (n,%)	11 (10.3%)
HbA1c %	5.8 (4.98-9)
Hypertension (n,%)	42 (39.3%)
Treatment for hypertension (n,%)	39 (36.4%)
LVMI (g/m^2)	108.5 (94-121)
LV concentric hypertrophy (n, %)	32 (29.9 %)
Aortic calcifications (n, %)	40 (37.4%)
BPs (mm Hg)	127.7 (110-160)
BPd (mm Hg)	79.7 (65-100)
Cholesterol (mg/dl)	191.6 (120-240)
HDL-Cholesterol (mg/dl)	62.9 (30-86)
Statin treatment	49 (45.8%)
Carotid atheromas	38 (35.5%)
Premature PAD in relatives	27 (25.2%)
Ejection fraction (%)	51.6 (40-64)
Ea (mmHg/mL)	2.07 (1.73-2.42)
Serum creatinine (mg/dl)	1.03 (0.5-2)
eGFR (ml/min/1.73 m^2)	75.6 (22-112)

Table 1. *Baseline data of recruited patients*
Variables are summarized as median and miminum-maximum value for continuous data and as number and percent for nominal variables, BPs = systolic blood pressure, BPd = diastolic blood pressure, Ea = arterial elastance, PAD = peripheral artery disease, LVMI = left ventricular mass index, HbA1c = glycosylated hemoglobin, eGFR = estimated glomerular filtration rate

VA-IMT (years)	65.5 (40-106)
VA-RF (years)	62.9 (37-88)
Biological age	57 (34-88)

Table 2. *Calculated vascular age*



Presence of *atherosclerotic* plaques in the carotid arteries were also recorded.

Estimation of vascular age

VA, according to cardiovascular risk factors (VA-RF) was estimated using Framingham score, taking into account: age, sex, smoking status, systolic blood pressure, treatment for hypertension, diabetes, TC, HDL-C[11]. We choose to use for simplicity Framingham score, instead of SCORE scale.

Furthermore, VA was estimated by assessing IMT (VA-IMT), using the calculator developed by Lian Engelen[22].

Arterial echo-Doppler assessment

A standard duplex ultrasound system with high resolution B-mode imaging, pulsed Doppler, spectral wawe form analysis and color flow Doppler imaging was used for scanning the lower extremity arteries (common femoral artery, superficial femoral artery, profound femoral artery, popliteal artery) using a 7,5 MHz linear transducer (Hitachi Aloka F37 ultrasound system). Velocities (peak systolic velocity) and other flow disturbancies were noted.

Plaque was defined as a focal structure protruding for at least 0,5 mm into the lumen or reaching a thickness more than 50% of the surrounding intima[30]. The 20 mm segment of the common femoral artery proximal to the bifurcation of the femoral tree was examined. In all cases, images were recorded in longitudinal and transverse planes. Angle-corrected spectral Doppler waveform and peak-systolic velocity measurements obtained from longitudinal images were recorded proximal to, at and distal to the suspected stenosis[28].

The significant peripheral arterial stenosis (PAS) was defined as more than two fold increase of Doppler systolic velocity across the stenosis This estimation is based on increase in peak systolic velocity of 100% with respect to the arterial segment above the stenosis, with a sensitivity 80% and specificity 93%[31].

Echocardiographic study

A comprehensive echocardiography was performed using Hitachi Aloka F 37 ultrasound system. We estimated left ventricular mass (LVM) according to the formula: LVM =0.8× [1.04{(IVSd + PWTd + LVIDd)^3–LVIDd^3}] + 0.6 g, where IVSd is diastolic diameter of interventricular septum, PWTd is diastolic diameter of posterior wall and LVIDd is diastolic diameter of the left ventricle. LVM was divided by body surface area (BSA) to obtain left ventricular mass index (LVMI). LV hypertrophy was defined as LV mass greater than 111 g/m^2 in men and greater than 106 g/m^2 in women The relative wall thickness (RWT) = (IVSd + PWTd)/LVIDd and considered increased if >0.42[32]. LV

Variable	Developed significant PAS (n=35)	Did not developed significant PAS (n=71)	p-value
Gender (male, n,%)	29 (39.2%)	45 (60.8%)	0.04
Age (years)	62.6 ± 10.6	54.4 ± 9.9	0.001
Smoking (n,%)	35 (43.7%)	45 (56.3%)	<0.001
Pack-years of smoking (mean ± SD)	25.6 ± 20.7	10.7 ± 15.5	<0.001
Diabetes (n,%)	12 (54.5%)	10 (45.5%)	0.016
Diabetes more than 10 years duration (n,%)	11 (100%)	0 (0%)	<0.001
HbA1c %	6.49 ± 1.11	5.55 ± 0.59	<0.001
Hypertension (n,%)	25 (59.5%)	17 (40.5%)	<0.001
Treatment for hypertension (n,%)	23 (59%)	16 (41%)	<0.001
LVMI (g/m^2)	110.07 ± 6.29	107.85 ± 7.38	0.13
LV concentric hypertrophy (n, %)	13 (40.6 %)	19 (59.4%)	0.27
Aortic calcifications (n, %)	16 (40%)	24 (60%)	0.23
Serum creatinine (mg/dl)	1.04 ± 0.29	1.03 ± 0.15	0.85
eGFR (ml/min/1.73 m^2)	76.77 ± 20.22	75.11 ± 15.52	0.64
BPs (mm Hg)	130 ± 11.9	126 ± 8.24	0.08
BPd (mm Hg)	80 ± 8.7	79 ± 8.61	0.81
Cholesterol (mg/dL)	203.4 ± 16.5	185.8 ± 21.3	<0.001
HDL-Cholesterol (mg/dL)	53.2 ± 9.7	67.7 ± 8.5	<0.001
Statin treatment (%)	28 (57.1%)	21 (42.9%)	<0.001
Carotid atheromas (%)	34 (89.5%)	4 (10.5%)	<0.001
Premature PAD in relatives (%)	10 (37%)	17 (63%)	0.60
Ejection fraction (%)	52.5 ± 5.7	51.2 ± 3.03	0.12
Ea (mmHg/mL)	2.16 ± 0.16	2.03 ± 0.15	0.001
VA-IMT	72.6 ± 16.9	62.1 ± 10.01	<0.001
VA-RF	74.2 ± 12.8	57.4 ± 9.5	<0.001

Table 3. *Comparative data of recruited pts between pts that did and did not developed significant PAS*

BPs = systolic blood pressure, BPd = diastolic blood pressure, Ea = arterial elastance, VA-IMT = vascular age calculated by intima-media thickness. VA-RF = vascular age calculated by cardiovascular risk factors. PAD = peripheral artery disease, LVMI = left ventricular mass index, HbA1c = glycosylated hemoglobin, eGFR = estimated glomerular filtration rate

Variable	Odds ratio	95% CI	p-value
Pack-years of smoking	**1.11**	**1.03-1.21**	**0.006**
Ea	7945.72	0.83-75398	0.055
VA-RF	1.03	0.81-1.30	0.77
VA-IMT	1.03	0.93-1.14	0.50
Cholesterol	1.03	0.98-1.08	0.15
Age	1.02	0.78-1.33	0.87
HDL-cholesterol	**0.75**	**0.65-0.88**	**0.001**
HbA1c %	**9.05**	**1.46-56.19**	**0.018**
Carotid atheromas	**921.60**	**34.80-24404.81**	**<0.001**

Table 4. *Logistic regression with event at follow-up as dependent variable (R squared value =0.56)*

Ea: = arterial elastance, HbA1c = glycosylated hemoglobin, VA-IMT = vascular age calculated by intima-media thickness, VA-RF = vascular age calculated by cardiovascular risk factors

geometry was assessed from LVMI and RWT values[32, 33], including normal geometry with normal LVMI and normal RWT, concentric remodeling with normal LVMI and increased RWT, concentric hypertrophy with increased LVMI and increased RWT, and eccentric hypertrophy with increased LVMI and normal RWT. LV ejection fraction was calculated using the biplane Simpson method and stroke volume (SV) was calculated as the product of LV outflow tract velocity time integral, obtained in pulsed Doppler from the apical 5 chamber view and LV outflow cross-sectional area, measured in parasternal long axis view. We noticed also the presence of *aortic calcification* (AC).

Assessment of arterial elastance

Arterial elastance (Ea) was calculated as the ratio of end-systolic pressure, estimated noninvasively as 0.9 X systolic blood pressure, to SV[26,34].

Follow-Up

Patients were followed up for 4.5 years ± 3 months. The primary end-point was the occuring of significant PAS, defined as a twofold increase Doppler velocity across the stenosis.

Statistical Analysis

Continuous variables were summarized as median and minimum-maximum value. Nominal variables were expressed as number and percent. In order to assess differences between groups, non-parametric Mann-Whitney U tests or Fisher-exact tests were performed (where appropriate). In order to verify the independent association between the significant variables with the outcome, a binary logistic regression was performed. IBM SPSS (version 20) for Windows was used for statistical analysis.

Results

Baseline characteristics of patients

Table 1 displays the demographic and clinical characteristics of the pts in the study group. They were young patients (median age 57 years), predominantly men (69,8%). Less than 40% had high blood pressure (39.3%), 36.4% had treated hypertension. The median LVMI was 108.5 g/m^2 and concentric hypertrophy was present in 29.9 % of pts. Pts with hypertension had LVMI of 113.12 g/m^2 and those without, had 105.6 g/m^2.

Diabetes mellitus (DM) was present in 20.6 % of patients, majority treated (88%). Among them, 10.3% had more than 10 years duration of DM. Median HbA1c was 5.8%. The majority of the pts are smokers (75.5%). Mean pack-years of smoking was 29.8. During the 4.5 years follow-up, 31.25% quit smoking. The cardiac performance was good - the median FE was 51.6%. The median TC concentration was 191.6 mg/dl and median HDL-C level was 62.9 mg/dl. 45.8% of the pts received treatment with statins. Premature atherosclerotic PAD in relatives was present in 25.2% of the patients, and carotid atheromas (CA) in 35.5%. AC were found in 37.4% of the patients. Median arterial elastance was 2.07 mmHg/ml. Median serum creatinine was 1.03 mg/dl and estimated glomerular filtration rate (eGFR) was 75.6 ml/ml/1.73 m^2.

In table 2 the calculated values of VA are presented. Median value of the VA calculated by IMT (VA-IMT) was 65.5 years and the median vascular age calculated by cardiovascular risk factors (VA-RF) was 62.9 years.

After 4.5 years of follow-up, 35 (32.7%) pts developed significant PAS. 22 pts had popliteal artey stenosis (62.85%) and 13 pts had superficial femoral artery stenosis



(37.15%). During the 4.5 years follow-up, 25 pts (31.25%) quit smoking.

Characteristcs of patients that developed significant PAS

Median age of pts who developed significant PAS at follow-up was 62.6 years and 39.2% were males. Individuals who developed stenosis tended to be smokers, older, receiving hypertensive and statin therapy, having DM, especially more than 10 years duration, having dyslipidemias and CA. They all had higher VA-IMT and VA-RF values compared to patients that did not develop PAS. Median value of VA-IMT was 72.6 years and VA-RF was 74.2 years ($p<0,001$ for both parameters). They also had significantlly higher values of Ea, with median value of 2.16 mmHg/ml.

There were no statistically significant differences between the two groups in cardiac performance (median FE=52.5%), LV mass (median LVMI = 110.07 g/m^2), percentage of LV concentric hypertrophy (40.6%), presence of AC (40%), premature PAD in relatives (37%), renal function (median eGFR = 76,77 ml/min/1.73 m^2) and systolic and diastolic blood pressure values (median values 130/80 mmHg).

The parameters of pts who developed significant PAS , compared with those who did not are shown in Table 3.

In order to verify the independent association between the significant variables with the outcome, a logistic regression was performed. Pack-years of smoking, HbA1c %, presence of CA and HDL-C were independently associated with the significant PAS progression (table 4). Ea did not reach independent prediction value, but was at the limit of statistical significance. ($p=0.055$)

From the 25 pts which quit smoking, only 6 developed significant PAS (24%), as compared to 29 (52.7%) from the 55 pts which remained active smokers ($p=0.016$).

Discussion

Analysing our data, we demonstrated that many factors are involved in PAD progression. Smoking is one of the main contributors, in concordance with data in the literature[9]. Although smoking significantly influenced PAD progression, at logistic regression it did not seem to have independent prediction value, this beeing evident only for the intensity of smoking, as measured by pack-years of smoking. On the other hand, among the patients who quit smoking during follow-up, progression of PAD was significantly lower. It is known that a longer period of smoking cessation showed a lower risk of all atherosclerotic diseases. Nevertheless, some adverse effects of smoking, such as pro-thrombotic state and endothelial dysfunction, are quickly reversible after

smoking cessation[35, 36]. We demonstrated that even after 4.5 years of smoking cessation there is a measurable effect on progression of PAD. Quitting smoking is very difficult to accomplish. We successfully used the concept of early vascular ageing (EVA)[13] in communication strategy with the patients, obtaining a high rate of smoking cessation (31.25%). In order to achieve this result, we presented to the pts VA, as the age of the arteries, making the abstract concept of cardiovascular risk more easily understood by pts. We used for estimation of VA two methods, one based on cardiovascular risk scores and another by direct measurement of carotid IMT. VA, calculated by both methods, was higher than biological age.

It was demonstrated that IMT correlates with atherosclerosis and has a good predictive value for cardiovascular events[15, 16, 17]. The increase of IMT with age was well documented in different age groups[37]. But the clinical value of IMT as contributor to cardiovascular risk was discarded from recent guidelines[38].

One explanation could be the fact that increase IMT comprises thickening of both intimal and medial layers and these may be due to different factors. It is possible that the influence of certain factors on thickening of the two layers could be different. This hypothesis was tested in a longitudinal study of the twins UK cohort. It was suggested that genetic and cardiovascular risk factors contribute differently to the progression of vascular changes[39]. The media layer suffers degenerative changes of extracellular matrix, with elastin fracture[23], which are probably genetically determined, whereas thickening of the intima is more influenced by environemental factors[40]. From the ultrasound imaging of the carotid artery we are unable to distinguish whether changes in wall thickness are due to atherosclerosis or vascular smooth muscle hypertrophy, because the measurement of IMT takes into account the combination of intima and media layers. The study group included subjects of older age which were probably exposed to environmental risk factors for a longer period of time, favorising thickening of the intima layer. The possibility of separatelly measurement of the two layers could provide in the future a better understanding of arterial impairment.

The media layer changes are reflected in arterial stiffness increase. Arterial stiffness was considered an earlier process in vascular pathology during the life course than atherosclerosis and this idea led since 2008 to the development of the concept of EVA[13]. It was demonstrated that arterial stiffness increases with age. Benetos, using PWV assessment, found an annual increases of 0.08 and 0.15 m/s in 296 normotensive and 187 hypertensive subjects respectively over a 6 year follow-up[41]. More recently, in the Multi-Ethnic Study of Atherosclerosis, the aortic wall thickness was evaluated and an increase with age was found[42]. A large number of studies have demonstrated an association of arterial stiffness with cardiovascular events[43,44,45].

We evaluated in our study Ea as a parameter indicating aortic stiffness[25, 26] and we found a statistically significant increase in patients who developed significant PAS, compared to those who did not (2.16 mmHg/ml vs 2.03 mm Hg/ml, p =0.001). However, we were not able to demonstrate an independent predictive role of Ea for the progression of PAD.

In our study, diabetes was associated with PAD progression, bu not in logistic regression, where only HBb A1c was found to be an independent predictor. It was demonstrated



that more severe or longstanding diabetes mellitus seems to be more strongly related to PAD. In the Hoorn Study, it was shown that known diabetes mellitus was associated with PAD in multivariable analysis, whereas newly diagnosed diabetes mellitus was only of borderline significance, and impaired glucose tolerance was not associated with PAD[46].

Despite its strong association with PAD, but because of its lower prevalence in populations when compared with other traditional risk factors, the population attributable fraction of type-2 DM for incident PAD was estimated only at 14% in a longitudinal study on US professionals[47].

It was previously demonstrated that subjects with PAD have also been shown to have an elevated prevalence of carotid artery stenosis[48]. In our study, just the presence of CA, without significant stenosis, was stongly correlated with PAD progression.

Total cholesterol value was significant for PAD progression in univariate analysis but dropped out of multivariable model, in which only HDL-C was statistically significant. It is unclear whether TC is the strongest independent risk factor for PAD; in one comparison of pts with PAD with healthy controls, it was found that mean TC did not differ significantly, whereas triglycerides, very low-density lipoprotein cholesterol, low-density lipoprotein cholesterol, HDL-C, and the TC/HDL-C ratio all did[49].

We found a significant higher blood pressure in patients with PAD progression, but without independent predictive value at multivariate analysis. Most other large, population-based studies have also found a significant, independent association of hypertension or systolic blood pressures with PAD[50].

Although the relative risks associated with hypertension are modest in some studies, its high prevalence, particularly among older pts, makes it a significant contributor to the total burden of PAD in the population[51].

We did not find any correlations between PAD progression and renal function, LV mass, LV systolic function, AC and premature PAD in relatives. It is possible that the last was underdiagnosed, because of ambiguous patient symptoms, although the global prevalence of premature atherosclerotic lower extremity PAD has gradually risen. Patients with premature PAD tend to have an aggressive vascular disease process, a high burden of cardiovascular risk factors, and other concomitant atherosclerotic vascular diseases like coronary artery disease[52].

Conclusions

We demonstrated that some of the classical cardiovascular risk factors were involved in PAD progression: smoking, dyslipidemia, diabetes, carotid atherosclerosis. At multivariate analysis the independent

variables associated with PAD progression were pack-years of smoking, glycosilated hemoglobin and HDL-cholesterol values and presence of carotid atheromas.

VA assessed by cardiovascular risk scales and by directly measured IMT both predict the progression of PAD, as did arterial elastance. None of them had independent prediction value for the outcome. Nevertheless, VA was usefull in comunicating with the patients, influencing their perception of disease progression and adherence to therapy.

VA, as the age of the arteries, has made the abstract concept of cardiovascular risk more easily understood by patients.

Nowadays, preventive medicine should be based on a patient centred approach, where the therapeutic decisions are taken in collaboration with the patient, who is empowerd to take part in the management of his health state. Explaining to the patient the abstract concept of cardiovascular risk is sometimes difficult. However, the idea of VA as the age of his arteries is something more easily understood by all patients, improving patient compliance with therapeutic decisions.

We think that these findings will improve shared decision making in patients with PAD. It is wellknown, for instance, the difficulty of adopting a healthy lifestyle, eg smoke cessation in this pacients. The concept of VA is more easily understood and valued by the patient in estimating the future progression of his arterial disease. We hope that these would contribute to an enhanced adherence to medical advice. We have shown that even after 4.5 years of smoking cessation there was a measurable effect on PAD progression.

Limitations

The present study has several limitations. The small number of patients included in the study is one important limitation. Another limitation is the relatively short 4.5 year follow-up period.

References

1. Fowkes FG, Rudan D, Rudan I, Aboyans V, Denenberg JO, McDermott MM, Norman PE, Sampson UK, Williams LJ, Mensah GA, Criqui MH. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. Lancet. 2013;382:13291340. doi: 10.1016/S0140-6736(13)61249-0.)

2. Michael H. Criqui, Victor Aboyans. Epidemiology of Peripheral Artery DiseaseCirc Res. 2015;116:1509-1526. DOI: 10.1161)

3. Althouse AD, Abbott JD, Forker AD, Bertolet M, Barinas-Mitchell E, Thurston RC, Mulukutla S, Aboyans V, Brooks MM; BARI 2D Study Group. Risk factors for incident peripheral arterial disease in type 2 diabetes: results from the Bypass Angioplasty Revascularization Investigation in type 2 Diabetes (BARI 2D) Trial. Diabetes Care. 2014;37:13461352. doi: 10.2337/dc13-2303),

4. Joosten MM, Pai JK, Bertoia ML, Rimm EB, Spiegelman D, Mittleman MA, Mukamal KJ. Associations between conventional cardiovascular risk factors and risk of peripheral artery disease in men. JAMA. 2012;308:16601667. doi: 10.1001/jama.2012.13415.)

5. Nicoloff AD, Taylor LM Jr, Sexton GJ, Schuff RA, Edwards JM, Yeager RA, Landry GJ, Moneta GL, Porter JM; Homocysteine and Progression of Atherosclerosis Study Investigators. Relationship between site of initial symptoms and subsequent progression of disease in a prospective study of atherosclerosis progression in patients receiving long-term treatment for symptomatic peripheral arterial disease. J Vasc Surg.2002;35:3846).

6. Bird CE, Criqui MH, Fronek A, Denenberg JO, Klauber MR, Langer RD. Quantitative and qualitative progression of peripheral arterial disease by non-invasive testing. Vasc Med. 1999;4:1521.)

7. Kennedy M, Solomon C, Manolio TA, Criqui MH, Newman AB, Polak JF, Burke GL, Enright P, Cushman M. Risk factors for declining anklebrachial index in men and women 65 years or older: the Cardiovascular Health Study. Arch Intern Med. 2005;165:18961902. doi: 10.1001/archinte.165.16.1896.)

8. Jonason T, Bergström R. Cessation of smoking in patients with intermittent claudication. Effects on the risk of peripheral vascular complications, myocardial infarction and mortality. Acta Med Scand. 1987; 221:253260.)

9. Ning Ding, MBBS, SCM,a Yingying Sang, MS,a Jingsha Chen, MS J Cigarette Smoking, Smoking Cessation, and Long-Term Risk of 3 Major Atherosclerotic Diseases Am



Internal Medicine

Original Papers

Coll Cardiol 2019;74:498507)

10. Jose´ I. Cuende, Natividad Cuende, and Javier Calaveras-Lagartos. How to calculate vascular age with the SCORE project scales: a new method of cardiovascular risk evaluation European Heart Journal (2010) 31, 23512358, doi:10.1093/eurheartj/ehq205)

11. D'Agostino RB Sr, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, Kannel WB. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation 2008;117: 743753.)

12. Law MR,Wald NJ, Morris JK. The performance of blood pressure and other cardiovascular risk factors as screening tests for ischaemic heart disease and stroke -J.Med.Screen (2004) 11:3-7.doi:10.1177/ 096914130301100102

13. P M Nilsson. Early vascular ageing A concept in development. European Endocrinology. 2015; 11(1):26-31

14. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. Circulation 1986; 74:13991406.

15. Peters SA, Grobbee DE, Bots ML. Carotid intima-media thickness: a suitable alternative for cardiovascular risk as outcome? Eur J Cardiovasc Prev Rehabil 2011;18: 167174.

16. M Rosvall, L Janzon, G Berglund, Incidence of stroke is related to carotid IMT even in the absence of plaque. Atherosclerosis. 2005 Apr;179(2):325-31

17. Matthias W Lorenz , Stefan von Kegler, Helmuth Steinmetz, Carotid intima-media thickening indicates a higher vascular risk across a wide age range: prospective data from the Carotid Atherosclerosis Progression Study (CAPS) Stroke. 2006 Jan;37(1):87-92

18. Den Ruijter HM, Peters SA, Anderson TJ, et al.. Common carotid intima-media thickness measurements in cardiovascular risk prediction: a meta-analysis. JAMA 2012;308:796803.

19. Sanne A E Peters, Hester M den Ruijter, Michiel L Bots, Karel G M Moons. Improvements in risk stratification for the occurrence of cardiovascular disease by imaging subclinical atherosclerosis: a systematic review. - Heart 2012;98:177e184. doi:10.1136/heartjnl-2011-300747)

20. Nagai Y, Metter EJ, Earley CJ, Kemper MK, Becker LC, Lakatta EG, et al. Increased carotid artery intimal-medial thickness in asymptomatic older subjects with exercise-induced myocardial ischemia. Circulation 1998; 98:1504-9.

21. Peter Willeit, Lena Tschiderer, et al. Carotid Intima-Media Thickness Progression as Surrogate Marker for Cardiovascular Risk: Meta-Analysis of 119 Clinical Trials Involving 100,667 Patients. Circulation. 2020;142:621642

22. Lian Engelen, Isabel Ferreira, Coen D. Stehouwer. Reference intervals for common carotid intima-media thickness measured with echotracking: relation with risk factors. European Heart Journal (2013) 34, 23682380 doi:10.1093/eurheartj/ehs380

23. Carlo Palombo, Michaela Kozakova. Arterial stiffness, atherosclerosis and cardiovascular risk: Pathophysiologic mechanisms and emerging clinical indications.. Vascular Pharmacology (2015), doi: 10.1016/j.vph.2015.11.083

24. Cavalcante JL, Lima JA, Redheuil A, Al-Mallah MH. Aortic stiffness: current understanding and future directions - J Am Coll Cardio (2011)57:1511-22. doi:10. 1016/j.jacc.2010.12.17

25. Munakata M. Brachial-ankle pulse wave velocity in the measurement of arterial stiffness: recent evidence and clinical applications. Curr Hypertens Rev. (2014) 10:4957. doi: 10.2174/157340211001141111 160957

26. Kelly RP, Ting CT, Yang TM, Liu CP, Maughan WL, Chang MS, et al. Effective arterial elastance as index of arterial vascular load in humans. Circulation 1992;86:513-21.

27. Chen C-H, Nakayama M, Nevo E, et al. Coupled systolic-ventricular and vascular stiffening with age: implications for pressure regulation and cardiac reserve in the elderly. J Am Coll Cardiol 1998;32:1221-7.

28. AIUM -The American Institute of Ultrasound Medicine Practice Parameter -Peripheral Arterial Ultrasound 2014

29. Iana Simova Intima media thickness; appropriate evaluation and proper measurement .e-Journal of Cardiology Practice vol 13, No21 -05 May 2015

30. Laclaustra M, Casasnovas J, Fernandez A- Femoral and Carotid Subclinical Atherosclerosis Association with Risk Factors and Coronary Calcium The AWHS Study - Journal of The American College of Cardiology, vol 67 No11, 2016, http//dx.doi.org/10.1016/jacc.2015.12.056

31. D Sacks1, M L Robinson, D L Marinelli, G S PerlmutterPeripheral arterial Doppler ultrasonography: diagnostic criteria J Ultrasound Med. 1992 Mar;11(3):95-103. doi: 10.7863/jum.1992.11.3.95.

32. Marwick TH, Gillebert TC, Aurigemma G, Chirinos J, Derumeaux G, Galderisi M, et al. Recommendations on the use of echocardiography in adult hypertension: a report from the European Association of Cardiovascular Imaging (EACVI) and the American Society of Echocardiography (ASE). J Am Soc Echocardiogr. 2015;28(7):72754.].

33. Silangei LK, Maro VP, Diefenthal H, Kapanda G, Dewhurst M, Mwandolela H, et al. Assessment of left ventricular geometrical patterns and function among hypertensive patients at a tertiary hospital, Northern Tanzania. BMC Cardiovasc Disord. 2012;12:109

34. Chantler PD, Lakatta EG, Najjar SS. Arterial-ventricular coupling: mechanistic insights into cardiovascular performance at rest and during exercise. J Appl Physiol 2008;105:13421351.3:

35. Camplain R, Meyer ML, Tanaka H, et al. Smoking behaviors and arterial stiffness measured by pulse wave velocity in older adults: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Hypertens 2016;29:126875.

36. McEvoy JW, Nasir K, DeFilippis AP, et al. Relationship of cigarette smoking with inflammation and subclinical vascular disease: the Multi-Ethnic Study of Atherosclerosis. Arterioscler Thromb Vasc Biol 2015;35:100210.)

37. Stein JH, Douglas PS, Srinivasan SR, Bond MG, Tang R, Li S, et al. Distribution and cross-sectional age-related increases of carotid artery intima-media thickness in young adults: The Bogalusa Heart Study. Stroke. 2004;35:27827.

38. 2018 ESC/ESH Guidelines for the management of arterial hypertension, 2018 ESC/ESH Guidelines for the management of arterial hypertension)

39. Marina Cecelja, Benyu Jiang, Louise Keehn, et al. Arterial stiffening is a heritable trait associated with arterial dilation but not wall thickening: a longitudinal study in the twins UK cohort. European Heart Journal (2018) 39, 22822288

40. Hayward S. C., Adji A, O'Rourke M. Arterial stiffening and arterial dilation as heritable traits caused by defective vital rubber? European Heart Journal (2018) 39, 22892290

41. Benetos A, Adamopoulos C, Bureau JM, Temmar M, Labat C, Bean K, Thomas F, Pannier B, Asmar R, Zureik M, Safar M, Guize L. Determinants of accelerated ,progression of arterial stiffness in normotensive subjects and in treated hypertensive subjects over a 6-year period, Circulation 2002;105:1202-1207

42. Liu CY, Chen D, Bluemke DA, Wu CO, Teixido-Tura G, Chugh A, Vasu S, Lima JA, Hundley WG. Evolution of aortic wall thickness and stiffness with atherosclerosis: long-term follow up from the multi-ethnic study of atherosclerosis. Hypertension 2015;65:10151019.

43. Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM, Vita JA,Levy D, Benjamin EJ. Arterial stiffness and cardiovascular events: the Framingham Heart Study. Circulation 2010; 121:505-511.

44. Ben-Shlomo Y, Spears M, Boustred C, May M, Anderson SG, Benjamin EJ,Boutouyrie P, Cameron J, Chen CH, Cruickshank JK, Hwang SJ, Lakatta EG, Laurent S, Maldonado J, Mitchell GF, Najjar SS, Newman AB, Ohishi M, Pannier B, Pereira T, Vasan RS, Shokawa T, Sutton-Tyrell K, Verbeke F, Wang KL, Webb DJ, Willum Hansen T, Zoungas S, McEniery CM, Cockcroft JR, Wilkinson IB. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. J Am Coll Cardiol 2014; 63:636-646.

45. van Sloten TT, Schram MT, van den Hurk K, Dekker JM, Nijpels G, Henry RM, Stehouwer CD. Local stiffness of the carotid and femoral artery is associated with incident cardiovascular events and all cause mortality: the Hoorn study. J Am Coll Cardiol 2014; 63:1739-1747.

46. Beks PJ, Mackaay AJ, de Neeling JN, de Vries H, Bouter LM, Heine RJ. Peripheral arterial disease in relation to glycaemic level in an elderly Caucasian population: the Hoorn study. Diabetologia. 1995;38:8696.)

47. Joosten MM, Pai JK, Bertoia ML, Rimm EB, Spiegelman D, Mittleman MA, Mukamal KJ. Associations between conventional cardiovascular risk factors and risk of peripheral artery disease in men. JAMA. 2012;308:16601667. doi: 0.1001/jama.2012.13415).

48. Pilcher JM, Danaher J, Khaw KT. The prevalence of asymptomatic carotid artery disease in patients with peripheral vascular disease. Clin Radiol.2000;55:5661. doi: 10.1053/crad.1999.0345.)

49. Mowat BF, Skinner ER, Wilson HM, Leng GC, Fowkes FG, Horrobin D. Alterations in plasma lipids, lipoproteins and high density lipoprotein subfractions in peripheral arterial disease. Atherosclerosis. 1997;131:161166.)

50. Criqui MH, Vargas V, Denenberg JO, Ho E, Allison M, Langer RD, Gamst A, Bundens WP, Fronek A. Ethnicity and peripheral arterial disease: the San Diego Population Study. Circulation. 2005;112:27032707. doi: 10.1161/CIRCULATIONAHA.105.546507.)

51. Meijer WT, Grobbee DE, Hunink MG, Hofman A, Hoes AW. Determinants of peripheral arterial disease in the elderly: the Rotterdam study. ArchIntern Med. 2000;160:29342938).

52. Anurag Mehta a , Devinder S. Dhindsa a , Ananya Hooda a , Premature atherosclerotic peripheral artery disease: An underrecogonized and undertreated disorder with a rising global prevalence, Trends in Cardiovascular Medicine, [m5G; June 26, 2020;12:46]).