„CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST
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PREDICTORS OF PERIPHERAL ARTERY DISEASE PROGRESSION IN RELATION TO THE CONCEPT OF VASCULAR AGE

PHD THESIS SUMMARY

Doctoral advisor:
PROF. UNIV. DR. ADRIANA ILIEŞIU

Doctoral student:
SAHLEAN (căs. POPA) RALUCA-DANA

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Thesis fundamentals

The fundamental issue addressed in this PhD thesis is the in-depth study of peripheral artery disease (PAD), whose prevalence and severity arer increasing all around the globe.

PAD is a manifestation of atherosclerosis, a distinct atherosclerotic syndrome, defined by aterial stenosis or occlusion, mainly those of the lower limbs. Nowadays, this condition is very widespread, being estimated that more than two hundred million people worldwide have PAD, with clinical manifestations ranging from paucisymptomatic or asymptomatic patients to patients with severe forms. [1]

The presence of PAD is associated with an increased risk of morbidity and mortality from coronary and cerebrovascular disease, due to the fact that the major risk factors (RF) for PAD are well-known RF of atherosclerosis, similar to those for coronary and cerebrovascular disease. [2].

If the RF for PAD development are extensively studied in the literature, those involved in the progression of PAD are less well defined.

Smoking is recognized as one of the strongest RF for PAD. [3] Smoking cessation among patients with intermittent claudication has been shown to improve various functional and physiological parameters related to PAD, also lowering cardiovascular mortality [4]. In this context, influencing the attitude of patients represents a challenge for modern medicine.

Current international and national recommendations emphasize adherence to therapeutic indications, and communication with patients in the spirit of shared medical decision-making (SMD).

Hypothesis

The research hypothesis on which the present study is based is the determination of the factors which may influence PAD progression. Another tested hypothesis is that the application of the SMD concept, simultaneously involvin both the doctor, and the patient, can significantly influence the patient's adherence to the therapeutic plan. In order to improve doctor-patient communication, we used the concept of vascular age (VA), to optimize the conveyance of the abstract concept of cardiovascular risk. From this perspective, the new idea of VA as the age of arteries is a concept easier to understand by all patients, improving their adherence to therapeutic decisions.

Objectives

The research objectives addressed in the PhD thesis are: the identification of the factors that can influence PAD progression, through the analysis of cardiovascular RF, comorbidities, subclinical atherosclerotic damage and the behaviors of a group of patients referred to the vascular exploration laboratory in a one-year interval, finding factors that have independent predictive value on PAD progression, evaluating the effect of effective, structured communication with the patient on his adherence to lifestyle changes.

Research methodology

The research method consisted of the following: clinical examination, laboratory tests, vascular Doppler ultrasound, echocardiography, completion of a lifestyle change intention questionnaire, explanatory talks with each patient. Patients were followed up for an mean period of 4.5 years.

The doctoral thesis includes 2 research projects:

Study I evaluates the progression of peripheral arterial lesions in patients with atherosclerosis in the lower limbs, without hemodynamically significant stenoses. Clinical and paraclinical factors involved in the progression of arterial stenoses have been analyzed and their predictive value has been assessed.

Study II evaluates patients' behavior and the possibility of influencing it by means of effective communication, in the spirit of doctor-patient SMD. Adherence to lifestyle changes, especially smoking cessation, was evaluated, studying the factors that could influence this decision, determining the predictive value of the analyzed factors for smoking cessation.

Chapter synthesis

Chapter I: General part

The general part of the thesis, structured in four chapters, illustrates data obtained by reviewing literature. We structured information about atherosclerosis, peripheral artery disease, vascular age, and doctor-patient communication.

Atherosclerosis

Atherosclerosis (ATS), a metabolic condition defined by the subintimal accumulation of fat, cholesterol, cellular degradation products and fibrin, leading to the formation of atheromatous plaques on the arterial walls, which become stiff, is an epidemic today, in the global context of a atherogenic diets and sedentarism. [5] The clinical picture of ATS is usually poor in symptoms in the initial phases, cardiovascular (CV) RF may be present, but when the stenoses exceed 70%, signs generated by insufficient blood flow may appear, depending on the affected territory: angina, neurological signs outbreak, intermittent claudication or signs of acute limb ischemia, abdominal pain, acute kidney failure etc. [6]

Conventional RF for ATS are hypertension (HT), impaired glucose tolerance/diabetes, dyslipidemia (high LDL-cholesterol, low HDL-cholesterol, high triglycerides), smoking, obesity, sedentary lifestyle, diet high in saturated fat etc. [7, 8, 9, 10, 11] Nonconventional markers of risk include elevated inflammatory markers such as high-sensitivity C-reactive protein, lipoprotein(a), homocysteine, hypercoagulability, direct imaging of the atheroma plaque (coronary calcium score, computed tomography computerized positron emission, magnetic resonance imaging, contrast ultrasound), genetic markers.

ATS can be non-invasively evaluated by ultrasonography, especially in the carotid arteries and in the arteries of the lower limbs, which are easily accessible.

Peripheral artery disease (PAD)

The main symptom of PAD is intermittent claudication, which is pain or a feeling of fatigue/discomfort, generally occurring when walking and disappearing at rest, located distal to the arterial stenosis. This symptom occurs after a certain walking distance, at a certain speed or incline of the road, and should be noted by the patient in order to monitor the stability of the disease, as there are dedicated questionnaires, for example the Edinburgh Claudication Questionnaire and the San Diego Claudication Questionnaire [12]. The clinical examination is

the basis of the assessment of patients with PAD, but the objective workup establishes the diagnosis. [13] Laboratory tests are recommended in the current PAD guideline in a progressive manner, starting with regular tests, up to complementary ones [13]. Diagnostic methods for PAD include the ankle-brachial index (ABI), Doppler ultrasonography (USD), digital subtraction angiography, computerized and magnetic resonance. USD includes B-mode ultrasound, pulsed, continuous, color and power Doppler, used to localize vascular lesions and quantify their extent using velocity criteria.

Vascular age (VA)

Vascular age (VA) is the apparent age of the vascular system, estimated by associated cardiovascular (CV) risk factors. VA translates the vascular damage that can sometimes appear prematurely, which emphasized the need for the concept of early vascular aging (EVA), representing arteriosclerosis at the medial level and early endothelial dysfunction, at the intimal level. [14] VA can be estimated by associated CV RF or by direct estimation methods, using intima-mean thickness (IMT). VA is useful in communicating with patients, influencing the perception of PAD progression and adherence to therapy, optimizing shared decision-making, mainly through smoking cessation. [15]

Doctor-patient communication.

Patient adherence refers to the degree to which a patient follows the doctor's recommendation. Adherence to the therapeutic regimen refers to both long-term adherence to medication and lifestyle change. [16] Adherence to treatment, according to the World Health Organization, is "the extent to which a person's behavior—taking medication, following a diet, and/or making lifestyle changes—corresponds to agreed-upon recommendations from a health care provider." [16]

Shared medical decision making (SMD)

Shared medical decision making (SMD) is a decision taken together with the patient, defined as an "approach where doctors and patients share information to make the best decision, and the patient is supported to consider all options, taking his preferences into account". [17]. SMD is currently gaining increasing importance in healthcare policies.

The second part of the thesis, dedicated to **personal contributions**, basically structures the obtained results.

Chapter II. Personal contributions

Study I - Predictors of peripheral artery disease progession

Working hypothesis and specific objectives

The present study tests the hypothesis that certain factors influence PAD progression. The purpose of the study was to follow and evaluate the progression of arterial lesions in patients who already have PAD on study inclusion. We investigated the clinical and paraclinical factors potentially involved in the progression of arterial stenoses, taking into account lifestyle, comorbidities, biological profile and patients' adherence to medical recommendations, assessed in the present study by smoking cessation. The aim of the study was to also evaluate the predictive value of the various studied parameters for the progression of PAD, to improve the management of these patients in the future. Another tested hypothesis is that the application of the SMD concept, with the simultaneous involvement of the doctor and the patient, can significantly influence the patient's adherence to the therapeutic plan.

The specific objectives were:

- Evaluation of the progression of lesions of the arteries of the lower limbs;
- Analysis of cardiovascular risk factors;
- Biological profile analysis;
- Evaluation of subclinical atherosclerotic damage in the territories of the aorta and carotid arteries, including arterial elastance;
- Analysis of comorbidities;
- Evaluation of patients' behavior, especially smoking cessation;
- Informing patients about premature vascular aging, using the concept of vascular age;
- Determining the predictive value of the analyzed factors for PAD progression.

Study population

The study included patients with insignificant arterial stenoses taken from the Vascular Surgery department of the "Prof Dr Gerota" Emergency Hospital between February 1, 2015 - December 31, 2015. During this period, 270 patients who presented for an arterial Doppler ultrasound of lower limbs were examined. All of these patients had atherosclerotic plaques in the arteries of the pelvic limbs, 164 of them having significant arterial stenoses. They were excluded from the study. In the end, 106 patients remained, aged 57.1 ± 10.8 years, of which 74 were men. These patients had insignificant arterial stenoses when evaluated on arterial

Doppler ultrasound at rest, and constituted the study group. They were further evaluated to monitor the eventual progression of the arterial stenoses, using duplex ultrasound and angio tomography. The study protocol was approved by the Local Ethics Committee in the hospital and all patients signed an informed consent.

Inclusion criteria:

- Patients with atherosclerosis in the lower limbs, without hemodynamically significant stenoses.

Exclusion criteria:

- Patients without atherosclerosis in the lower limbs;
- Patients with hemodynamically significant lower limb arterial stenoses, defined as an increase in velocity at the level of the stenosis to more than twice the velocity before the stenosis.

At inclusion, patients were evaluated by complete clinical examination, carotid Doppler ultrasound, echocardiography, femoral artery Doppler ultrasound, and biochemical tests.

The presence of CVRF, medical history, medication use, presence and duration of smoking, blood pressure (BP) values, the presence and age of diabetes, and the premature presence of atherosclerotic PAD in patients' relatives, characterized by its diagnosis before the age of 50, were evaluated. Total cholesterol (TC), HDL cholesterol (HDL-C), serum glucose, glycosylated hemoglobin (HbA1C), serum creatinine, estimated glomerular filtration rate (eGFR) were also determined.

Carotid arteries were evaluated using a Hitachi Aloka F37 machine with a 7.5 MHz linear transducer ultrasound in two-dimensional mode, recording the presence of atheroma plaques. IMT, representing the combined thickness of the intima and media was also assessed. The distance between the two echogenic lines on the far wall of the common carotid artery representing the lumen-intima and media-adventitum interface was measured. [18]

VA was estimated in two ways: classically, using cardiovascular risk factors (VA-RF), and by using IMT (VV-GIM). Considered RFs were age, sex, smoking, systemic BP, presence of antihypertensive treatment, presence of diabetes, and TC and HDL-cholesterol levels. [19] We chose to use the Framingham score instead of the SCORE scale for simplicity. VA was also estimated directly, by IMT evaluation, using a calculator recently developed by Lian Engelen. [20]

We used the concept of VA in the communication strategy with patients, in order to improve adherence to lifestyle change, especially smoking cessation. Patients filled in a lifestyle change intention questionnaire, the results of which will be presented in study II.

The arteries of the lower limbs were evaluated using a Hitachi Aloka F37 ultrasound system with a 7.5 MHz linear transducer in two-dimensional mode, color Doppler, pulsed Doppler. Common femoral arteries (CFA), deep femoral arteries (DFA), superficial femoral arteries (SFA), and popliteal arteries (PA) were scanned, recording the presence of atheroma plaques, peak systolic velocities, and other changes in arterial flow.

Atheroma plaque was defined as a focal structure protruding at least 0.5 mm into the lumen or with a thickness greater than 50% compared to the thickness of the adjacent intima. [21]

Confirmation of arterial lesions of the lower limbs was performed using a Toshiba Aquilon CXL 128 tomograph with 64 slices. The common femoral arteries (CFA), deep femoral arteries (AFP), superficial femoral arteries (AFS) and popliteal arteries (AP) were scanned, the three-dimensional reconstruction of the images was performed, the presence of atheroma plaques was recorded and the stenoses were quantified.

A comprehensive echocardiographic study was performed using a Hitachi Aloka F37 machine. Left ventricular mass (LVM) and its geometry, defined by relative wall thickness (RWT), were estimated. Left ventricular ejection fraction (EF) was calculated using the Simpson biplane method in the apical four-chamber and two-chamber sections.

Stroke volume (SV) was estimated as the product of the time-velocity integral of the LV ejection tract, obtained using pulsed Doppler in the five-chamber apical section, and the area of the LV ejection tract, measured in the long-axis parasternal section.

The presence of aortic calcifications (AC) was also recorded, in the parasternal long axis section, at the level of the aortic annulus and the ascending aorta. The presence of calcifications was also recorded at the aortic arch, in the suprasternal section.

Arterial elastance was calculated as the ratio of end-systolic arterial pressure (estimated noninvasively as 0.9 multiplied by the systolic BP value measured in the arm with a sphygmomanometer) and VBVS, estimated as the product of the time-velocity integral of the ejection tract of LV, obtained using pulsed Doppler in the five-chamber apical section and LV ejection tract area, measured in the parasternal long-axis section. [22, 23]

The **study variables** that were selected following patient evaluation were:

- Sex;
- Age;
- Smoking;
- Pack-years of smoking;
- Diabetes;
- Duration of diabetes for more than 10 years;
- Glycosylated hemoglobin;
- •Hypertension;
- Treatment for high blood pressure;
- Indexed left ventricular mass;
- Concentric hypertrophy of the left ventricle;
- The presence of aortic calcifications;
- Systolic blood pressure;
- Diastolic blood pressure;
- Total cholesterol;
- HDL-cholesterol;
- Treatment with statins
- The presence of carotid atheromas;
- Presence of premature peripheral arterial disease in relatives;
- Left ventricular ejection fraction;
- Arterial elastance;
- Creatinine;
- Estimated glomerular filtration rate;
- Vascular age estimated by risk factors;
- Vascular age calculated by measuring the intima-media index.

Patients were followed up annually, clinically and paraclinically. On the final visit, an arterial Doppler examination of the lower limbs was performed to assess arterial stenoses. On the final evaluation visit, the progression of PAD was monitored, by the occurrence of significant peripheral arterial stenoses (SAPS). According to the appearance of SAPS, the patients were divided into two groups, A and B. In those of group A, a progression of PAD was detected, with the appearance of SAPS, in those of group B, the disease did not evolve significantly, no SAPS appeared (NSAPS).

Statistical analysis was performed using IBM SPSS Statistics 20. Continuous variables were expressed as median and minimum and maximum values, as well as mean ± standard deviation (SD). Quantitative variables were expressed as number and percentage. Quantitative independent variables with non-parametric distribution were tested using the Mann-Whitney U test, and Spearman's rho correlation coefficient was used for correlations between them. Paired quantitative variables with parametric distribution were tested using the Paired Samples T-Test. Paired quantitative variables with non-parametric distribution were tested using the Related-Samples Wilcoxon Signed Rank Test. Binary logistic regression was used to verify the independent association between significant variables and events. A value of $p<0.05$ was considered statistically significant, and the confidence interval was 95%.

Results

Baseline characteristics of patients

Most patients are men (69.8%), the mean age is 57 years, there were 80 smokers in the group (75.5%), with the number of pack-years of smoking of 29.8±16.1, 22 patients had diabetes (20.6%), of which 11 patients had diabetes lasting more than 10 years (10.3%), glycosylated hemoglobin (HbA1c) had a mean value of 5.8%, 42 of patients were hypertensive (39.3%), of which 39 patients were receiving antihypertensive treatment (36.4%). The LV mass index was 108.5 g/m2, and 32 patients had concentric LV hypertrophy (29.9%). 40 patients had aortic calcifications (37.4%). Systolic BP was normal (127.7mmHg), diastolic BP was also normal (79.7mmHg). TC values were 191.6mg/dl, and HDL-c values were 69.9mg/dl. 49 patients had statin treatment (45.8%), and 38 patients had carotid atheromas (35.5%). Premature PAD in the relatives of the examined patients was present in 27 patients (25.2%). Renal function was mildly altered, with serum creatinine of 1.03 mg/dl and eGFR of 75.6 ml/min/1.73m2. Mean LV EF was 51.6%. Mean arterial elastance was 2.07 mmHg/ml.

Taking into account the presence of CVRF, VA was estimated based on the Framingham score, then calculated by the IMT-based algorithm as well.

The VA calculated using IMT was 65.5 years, and the VA estimated by CVRF was 69.2 years, higher compared to the biological age.

Patients' evolution

Patients were followed for 4.5 years ± 3 months. At the evaluation visit, the progression of PAD was monitored, by the appearance of SAPS. During this period, 35 (32.7%) patients

developed a significant PAD progression at duplex ultrasound, as follows: 22 patients developed popliteal artery stenosis (62.85%) and 13 patients developed superficial femoral artery stenosis (37, 15%).

Adherence to lifestyle change was also tracked. Thus, during the 4.5 years of follow-up, 25 people (31.25%) ceased smoking. Smoking cessation occurred after the first visit.

Characteristics of patients who developed significant peripheral arterial stenoses

We present in the following table (table 1) the characteristics of patients in whom we detected a progression of peripheral arterial disease (Group A), compared to patients in whom the disease did not evolve significantly (Group B).

Table 1. Comparative data of patients who developed (group A) or did not develop (group B) significant peripheral arterial stenoses. BPs – systolic blood pressure, BPd – diastolic blood pressure, HbA1c = glycosylated hemoglobin, eGFR = estimated glomerular filtration rate, Ea = arterial elastance, PAD = peripheral arterial disease; iLVM = LV mass index, VA-IMT = vascular age estimated by intima-media thickness. VA-RF = vascular age estimated by risk factors.

Variable	**Group A (n=35)**	**Group B (n=71)**	**p**
Sex (male, n,%)	29 (39.2%)	45 (60.8%)	0.04
Age (years)	**62.6 ± 10.6**	**54.4 ± 9.9**	**0.001**
Smokers (n,%)	**35 (43.7%)**	**45 (56.3%)**	**<0.001**
Pack-year (mean ± SD)	**25.6 ± 20.7**	**10.7 ± 15.5**	**<0.001**
Diabetes (n,%)	**12 (54.5%)**	**10 (45.5%)**	**0.016**
Diabetes lasting more than 10 tears (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**
iLVM (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**
Treatment with statins (%)	**28 (57.1%)**	**21 (42.9%)**	**<0.001**
Carotid atheroma (%)	**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 (years)	**72.6 ± 16.9**	**62.1 ± 10.01**	**<0.001**
VA-RF (years)	**74.2 ± 12.8**	**57.4 ± 9.5**	**<0.001**

By analyzing the evaluated parameters comparing patients with PAD progression during the 4.5 years of follow-up with those who remained stationary, we identified numerous factors that were significantly different between the two study groups.

These factors were:

- Age;
- Smoking;
- the number of pack-years of smoking;
- diabetes, regardless of its duration;
- the level of glycosylated hemoglobin;
- hypertension, treated or not;
- dyslipidemia – (value of total cholesterol and HDL-cholesterol), treated or not
- the presence of carotid atheroma;
- the value of arterial elasticity;
- the value of vascular age estimated by both risk factors and IMT measurement.

We further assessed the factors that may independently influence PAD progression by multivariate statistical evaluation to determine which of the analyzed factors might have independent predictive power. To verify the independent association between the variables studied and the endpoint constituted by the occurrence of significant peripheral arterial stenoses, a logistic regression was performed.

Table 2. Logistic regression. HbA1c = glycosylated hemoglobin

Variable	Odds ratio	95% CI	p
Pack-year	**1.11**	**1.03-1.21**	**0.006**
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 atheroma	**921.60**	**34.80-24404.81**	**<0.001**

Variables that qualified for independent association with significant PAD progression were:

- number of pack-years of smoking;
- level of glycosylated hemoglobin;
- presence of carotid atheroma;
- HDL-cholesterol level.

Arterial elastance did not reach the independent predictive value, but was borderline statistically significant (p=0.055).

Discussion

Analyzing the data from this study, we demonstrated that in the evaluated patient group there was a progression of PAD in some patients, similar to other data from the literature. Thus, Nicoloff et al. demonstrated progression of PAD, finding that over 5 years, 37% of patients with PAD had a significant worsening (≥0.15) of the ABI, while 22% of patients had clinical progression of PAD, manifested by a change in symptoms or by the appearance of an indication for surgical intervention [24]

In the current study it was demonstrated that several factors can be involved in the progression of PAD. Smoking is one of the main contributors, consistent with data from the specialized literature. [3] Smoking is recognized as one of the strongest risk factors for PAD.

In the present study it was shown that smoking significantly influenced the progression of PAD, but in logistic regression it did not have an independent predictive value. Smoking intensity, measured by pack-years of smoking, proved to be a more accurate indicator for predicting PAD progression. Quitting was more common in patients who smoked less, with the number of pack-years of smoking almost double in those who failed to quit. This result is consistent with data from the literature, which show that the rate of smoking cessation is higher in subjects who are not heavy smokers, although it is precisely in this category that smoking cessation would be most beneficial. [3] The question of the time interval after smoking cessation during which beneficial preventive effects can occur has been raised. Thus, it was found that a lower risk of PAD, coronary heart disease, and stroke was observed even within 5 years of quitting smoking [3]

In the present study it was shown that even after 4.5 years of smoking cessation, there is a measurable effect on reducing the progression of PAD.

Smoking cessation is very difficult to achieve. The concept of EVA [14] was successfully used in the communication strategy with patients, achieving a high rate of smoking cessation (31.25%) To get this result, we presented VA as the age of the arteries, making the abstract concept of cardiovascular risk easier for patients to grasp. We started from the idea that, nowadays, preventive medicine should be based on a patient-centered approach, where therapeutic decisions are made in collaboration with the patient, who should be involved in the management of his health condition. The influence of the VA concept on patients' adherence to lifestyle change is presented in extenso in study II.

Arterial stiffness (AS), produced by changes in the tunica media, could be involved in the progression of PAD. AS has been considered an earlier process in vascular pathology during life than atherosclerosis and this idea has led since 2008 to the development of the concept of EVA. [14]. AS has been shown to increase with age. Benetos, using pulse wave velocity (PWV) assessment, found an annual increase of 0.08 and 0.15 m/s in 296 normotensive and 187 hypertensive subjects, respectively, over a 6-year follow-up period. [25] AS is usually assessed by means of PWV, estimating the propagation speed of the arterial pulse. Another, more accessible, method of assessing AS is the measurement of effective arterial elastance (Ea), which describes in particular the characteristics of the aorta. It, introduced as a measure of cardiac afterload, [26] is also considered a measure of AS [27]. It is evaluated by calculating the ratio between the end-systolic pressure in the left ventricle and the stroke volume,

combining the average and pulsatile components of the afterload [26]. I chose this method considering it more accurate, because it also takes into account the BP value.

Ea was evaluated in the present study as a parameter indicating aortic stiffness [26, 27], the inverse of compliance, and found a statistically significant increase in the number of patients with increased elastance, who developed significant PAD, compared to those in whom PAD did not register a significant progression. (2.16 mm Hg/ml vs 2.03 mm Hg/ml, p=0.001). However, we could not demonstrate an independent predictive role of Ea for PAD progression.

In the present study, diabetes was associated with PAD progression, but not in logistic regression, where only HbA1c was found to be an independent predictor. Elevated Hb A1c may reflect the presence of uncontrolled diabetes.

Subjects with PAD have also been previously shown to have an increased prevalence of carotid artery stenoses [28]. In our study, the presence of carotid atheromatous plaques, even in the absence of significant stenoses, was strongly correlated with PAD progression, demonstrating the validity of the concept of multivascular atherosclerotic damage.

Increased TC was significant for PAD progression in univariate analysis, but in multivariate analysis only increased HDL-c was statistically significant, which was a surprising result. It is not clear whether TC is the strongest independent risk factor for PAD, and it is possible that other lipid fractions are more important. Thus, in a study that evaluated various lipid fractions in patients with PAD compared to healthy controls, it was found that the mean value of TC did not differ significantly between the two groups, while triglycerides, low and very low density lipoproteins, HDL -c and TC/HDL-c ratio are significantly different in patients compared to healthy subjects. [29]

In the present study, we found a significantly higher BP in patients with PAD progression, but without independent predictive value on multivariate analysis. Most other large, population-based studies also found a significant and independent association of hypertension (HTN) or systolic BP with PAD. [30] Although the relative risks associated with HTN are modest in some studies, its high prevalence, particularly among older patients, makes it a significant contributor to the total burden of PAD in the population. [31]

It is worth mentioning that relatively low TC values were recorded in the entire study group (191.6mg/dL), probably in the context of statin treatment, present in almost half of the patients. The same is true for the normal BP values recorded in the entire study group, in the

context of the antihypertensive treatment present in most known hypertensive patients (BP=127.7mmHg, BP=79.7mmHg).

We found no correlations between PAD progression and aortic calcifications, whereas carotid atheromas had a significant predictive value for PAD progression, which was a surprising result. Data from the literature highlight new complex phenomena involved in vascular calcifications, similar to those in bone remodeling, involving circulating calciprotein particles, mRNA, osteoclast membrane proteins (RANK) leading to the formation of bone-like tissue. [32]. Carotid atheromas, produced by the phenomena of atherosclerosis, are probably associated with the progression of PAD, while aortic calcifications, predominantly influenced by bone metabolism disorders, do not correlate with the evolution of PAD.

In the present study, no significant correlations were demonstrated between the progression of PAD and the presence of premature PAD in the patients' relatives. It is possible that the presence of PAD in relatives has been underdiagnosed, due to nonspecific symptoms of patients or asymptomatic disease. We did not have access to examination of patients' relatives, therefore ABI was not determined. Data from CVD and other concomitant atherosclerotic vascular diseases such as coronary artery disease [33].

Limitations of the study

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

Study II: Influence of the vascular age concept on patient adherence to lifestyle changes

Working hypothesis and specific objectives

The present study tests the hypothesis that good communication with the patient can influence their adherence to lifestyle change, focusing on discussions of the major influence of smoking on PAD and also on the concepts of CV risk and VA. It was also tested the hypothesis that it is feasible to involve patients in changing their lifestyle, by applying the concept of SMD, with the simultaneous contribution of the doctor and the patient.

Specific objectives:

• Assessment of patients' behaviors, especially intention to quit smoking

• Informing patients about premature vascular aging, using the concept of vascular age

• Evaluation of adherence declaratively, by completing a specific questionnaire

• Assessing factors that may influence adherence to lifestyle change

• Determining the predictive value of the analyzed factors for smoking cessation

• Evaluation of patient awareness of the effects of smoking.

The study population, inclusion and exclusion criteria were the same as in study I. In addition to monitoring the progression of arterial stenoses, the patients' attitude towards lifestyle change was actively monitored, with an emphasis on smoking cessation. The investigations performed were the same as in study I. The patients were informed about the results of these investigations and were subsequently asked to complete a questionnaire to measure opinions and behavior regarding their perception of the possibility of lifestyle change. The questionnaire included 10 questions, with answers ranging from 1 to 5 (1 = I strongly disagree, 5 = I strongly agree).

Questionnaire measuring attitude and intention towards lifestyle change, especially smoking cessation:

Table 3. Intent questionnaire - lifestyle changes

Lifestyle change intention questionnaire	Strongly disagree	Partially disagree	Neither agree, nor disagree	Partially agree	Strongly agree
1. Are you convinced that smoking affects the health of your arteries?	1	2	3	4	5
2. Did you know that the age of a man depends on the age of his arteries?	1	2	3	4	5
3. Are you convinced that you have to change your habits, giving up things you like?	1	2	3	4	5

4. Are you ready to give up at least one of these things, for example smoking?	1	2	3	4	5
5. Are you willing to replace smoking with something beneficial, for example exercise?	1	2	3	4	5
6. Do you think that quitting smoking will improve your health?	1	2	3	4	5
7. Did you know that quitting smoking can improve how long you can walk without pain?	1	2	3	4	5
8. Did you know that lower limb artery disease may be associated with myocardial infarction or stroke?	1	2	3	4	5
9. Would you like to become an example to your family that you have succeeded in quitting smoking?	1	2	3	4	5
10. Are you planning to make the change today?	1	2	3	4	5

Responses to this questionnaire were quantified as a lifestyle change adherence score (SASV) by dividing the sum of the scores calculated for each patient by 10 (the number of questions).

Results

The baseline characteristics of the patients were presented in detail in study I, which analysed the factors that influenced the progression of PAD.

SASV was on average 3.3±0.7. The present study focused on changing the patients' lifestyle. Thus, during the 4.5 years of follow-up, 25 people (31.25%) quit smoking. Other lifestyle changes were insignificant: no weight loss, increased physical activity, or dietary changes.

The patients were divided into two groups, which were analyzed:

- Group A – patients who quit smoking, 25 (31.25%);
- Group B – patients who continued to smoke, 55 (68.75%).

Comparative analysis of the parameters of the two groups of patients A and B – those who quit smoking, and those who continued to smoke

We present in the following table (table 8.3) the characteristics of patients who quit smoking (Group A), compared to patients who continued to smoke (Group B).

Table 4. Comparative data of patients who quit smoking (group A) or did not quit (group B). BPs – systolic blood pressure, BPd – diastolic blood pressure, HbA1c = glycosylated hemoglobin, eGFR = estimated glomerular filtration rate, Ea = arterial elastance, PAD = peripheral arterial disease; iLVM = LV mass index, VA-IMT = vascular age estimated by intima-media thickness. VA-RF = vascular age estimated by risk factors; SASV = lifestyle change adherence score.

Variable	**Group A (n=25)**	**Group B (n=55)**	**p**
Sex (bărbați, n,%)	17 (30.9%)	38 (69.1%)	0.92
Age (years)	56 (53 – 62)	55 (53-59)	0.7
Pack-year (mean ± SD)	**11.52 ± 17.4**	**23.02 ± 19.27**	**0.013**
Diabetes (n,%)	5 (29.4%)	12 (70.6%)	0.85
Diabetes lasting more than 10 tears (n,%)	1 (9.1%)	10 (90.9%)	0.15
HbA1c %	5.89 ± 0.85	5.97 ± 1.03	0.66
Hypertension (n,%)	10 (32.3%)	21 (67.7%)	0.87
Treatment for hypertension (n,%)	10 (34.5%)	19(65.5%)	0.63
iLVM (g/m^2) (mean ± SD)	107.05 ± 6.66	107.47 ± 7.25	0.8
LV concentric hypertrophy (n, %)	7 (28%)	18 (72%)	0.67

Aortic calcifications (n, %)	10 (34.5%)	19 (65.5%)	0.63
Serum creatinine (mg/dl) (mean ± SD)	1.06 ± 0.23	1.00 ± 0.21	0.32
eGFR (ml/min/1.73 m^2) (mean ± SD)	73.00 ± 18.52	78.56 ± 17.35	0.21
BPs (mm Hg) (mean ± SD)	130 ± 9.3	126.5 ± 10.34	0.14
BPd (mm Hg) (mean ± SD)	**82.8 ± 8.98**	**78.00 ± 8.22**	**0.02**
Cholesterol (mg/dL) (mean ± SD)	189 ± 19.94	193.58 ± 22.08	0.36
HDL-Cholesterol (mg/dL) (mean ± SD)	62.44 ± 9.3	61.54 ± 12.72	0.75
Treatment with statins (%)	10 (22.7%)	34 (77,3%)	0.06
Carotid atheroma (%)	8 (21.1%)	30 (78.9%)	0.061
Premature PAD in relatives (%)	6 (30%)	14 (70%)	0.88
Ejection fraction (%)(mean ± SD)	**49.68 ± 3.9**	**52.78 ± 4.36**	**0.003**
Ea (mmHg/mL) (mean ± SD)	2.07 ± 0.17	2.10 ± 0.15	0.44
VA-IMT (years) (mean ± SD)	**73.20 ± 15.39**	**63.32 ± 13.21**	**0.008**
VA-RF (years) (mean ± SD)	66.36 ± 13.1	62.96 ± 14.33	0.30
SASV	**3.5±0.8**	**3.1±0.6**	**0.042**

In addition to patients' intention to change their lifestyle, we also analyzed awareness of the assciation between smoking and PAD and between PAD and other vascular conditions.

Among the responses to the lifestyle change intention questionnaire, we additionally analyzed those from the first question " Are you convinced that smoking affects the health of your arteries?", to assess the degree of awareness of the link between smoking and PAD and those from question no. 8 "Did you know that lower limb artery disease can be associated with myocardial infarction or stroke?". Thus, we found that 35% of smokers did not know that smoking is related to PAD. We also found that 70% of smokers were unaware of the link between PAD and other cardiovascular events.

We further evaluated factors that may independently influence smoking cessation by performing a multivariate statistical analyss to determine which of the analyzed factors may have independent predictive power.

To verify the independent association between the studied variables and the smoking cessation endpoint, a logistic regression was performed.

The results are presented in table 5.

Table 5. Logistic regression. VA-IMT = vascular age estimated by intima-media thickness., EF = left ventcle ejection fraction, BPd – diastolic blood pressure. SASV = lifestyle change adherence score

Variable	**Odds Ratio**	**95% CI**	**p**
Pack-year	0.97	0.94-1.01	0.25
VA-IMT	**1.06**	**1.015-1.112**	**0.01**
EF %	0.90	0.77-1.06	0.24
BPd	1.05	0.97-1.13	0.21
SASV	**15.90**	**1.40-180.37**	**0.02**

Variables that qualified for independent association with smoking cessation were:

- vascular age estimated by intima-media thickness;
- lifestyle change adherence score.

Discussion

Analyzing the data from this study, we demonstrated that informing patients about EVA, with the help of the VA concept, contributed to patients' adherence to therapeutic indications regarding lifestyle change, influencing the decision to quit smoking.

Factors that significantly influenced smoking cessation in the present study, including as independent variables, were vascular age (p=0.01) and lifestyle change adherence score (p=0.02). The concept of EVA (87) was successfully used in the communication strategy with patients, achieving a high rate of smoking cessation (31.25%)

Adherence - respectively non-adherence to the treatment - is the result of a combination of factors, some of which are specific to the patient, and another part are determined by the drug/treatment. Last but not least, the doctor-patient relationship of trust is found among the factors with a positive effect on treatment adherence. [34].

In the current study we also assessed the degree of awareness of lifestyle change on their health status and the possibility of certain cardiovascular events.

For this reason, the questionnaire also included two general questions about the awareness of the link between smoking and PAD, as well as about the association of PAD with damage to other vascular territories. We found that although many patients are aware of the deleterious effects of smoking on lung cancer, many are unaware of the link between smoking and peripheral arterial disease. In the present study, 35% of patients were unaware of the link between smoking and their intermittent claudication pain. Also, most patients diagnosed with symptomatic or asymptomatic PAD ignore its association with other cardiovascular events. In the present study, 70% of patients were unaware of the connection between the presence of PAD and myocardial infarction or stroke. Data from the literature demonstrated a doubling of the frequency of coronary events, cardiovascular and total mortality at 10 years in patients with PAD. [35] Another study highlights, within 5 years from the diagnosis of PAD, the appearance in 20% of these patients of an acute myocardial infarction or stroke, with a mortality of 10 - 15%. [35]

Literature data abounds in information that unequivocally demonstrates the link between lifestyle and cardiovascular events. Smoking cessation is potentially the most effective of all cardiovascular event prevention measures, with substantial reductions in myocardial infarction recurrence and mortality. [36, 37]. Quitting smoking adds years to life free of cardiovascular events. The gains in CVD-free years are substantial at all ages, and the benefits are obviously even more substantial when other complications of smoking are taken into account. Even in heavy smokers, quitting smoking reduces the risk of cardiovascular disease within 5 years, although it remains higher than in nonsmokers. [38].

In the present study we showed that effective communication with patients can contribute to their beneficial decisions regarding their health status, in accordance with the concept of SMD.

Mutual medical decisions are based on understanding and respect. The doctor and the patient are equal partners and contribute to the decision with their experiences. The doctor comes with his medical experience and expertise, and the patient comes with his knowledge and personal experience.

However, some healthcare professionals express doubts about the effectiveness of SMD, claiming that patients do not want to be involved in decisions and some may make "bad" decisions. On the other hand, SMD is not considered practical given the time constraints of physicians. Although some physicians claim that they "already do it," data from patient experience surveys indicate that this is not the case. [39]. The two-way exchange of information, also taking into account information shared by patients about their values and

preferences, is what separates SMD from one-way patient education. When clinicians try to guess what patients value, they can often be wrong. [40]

With regard to smoking cessation, the current study demonstrated that several factors may be involved. Thus, the number of pack-years of smoking was lower in those who managed to quit. This result is consistent with data from the literature, which shows that the rate of smoking cessation is higher in subjects who are not heavy smokers, although it is precisely in this category that smoking cessation would be most beneficial. [3] However, we could not demonstrate an independent predictive role of smoking intensity on multivariate analysis.

Smoking, with an average of 7 million deaths per year, is currently the main preventable cause of death [41]. Among smokers who are aware of the harmful effects of smoking, 3 out of 4 people are interested in quitting. [42]. More recent data from the smoking cessation literature have shown that approximately two-thirds of smokers are interested in quitting, with over 50% reporting that they have made an attempt in the past year. [43]. However, only one in ten people managed to be successful, effectively quitting smoking within a year. [43].

Although there are drug treatments considered effective, such as nicotine replacement therapy (patches, gum, sublingual tablets, inhalers and nasal sprays) or drugs such as Bupropion and Varenicline, their effectiveness is not impressive. Patient education is more accessible, with educational methods having a higher cost-benefit ratio. [44]

General conclusions and original contributions

Conclusions

- The objectives of the present research were fully achieved, both regarding the identification of the factors involved in the progression of peripheral arterial disease, and the communication with the patient, in the spirit of the shared medical decision between the doctor and the patient, in order to improve patient adherence to therapeutic indications.
- The technical-economic advantages of our approach are as follows:
    - Identifying the factors involved in the progression of PAD facilitates the prevention of the evolution of this disabling disease, potentially avoiding expensive surgical interventions;
    - Effective communication with the patient, based on explanatory talks and the use of easier-to-understand concepts, such as vascular age, can improve his adherence to therapeutic indications, favoring an improvement in PAD.

- The technical-economic disadvantages of our approach are:
    - Increasing the duration of interaction with the patient, given the previously existing overload of the medical staff;
    - Insufficient training of medical personnel in communication strategies.
- Unsolved problems:
    - o Development of concrete protocols for communication with patients, based on appropriate training of the medical staff;
    - o Continuing the follow-up of evaluated patients, through a loyalty based on a protocol with their family physicians.
- Directiions for future research:
    - Research should be extended to the primary prevention of PAD, by early control of the risk factors involved in the occurrence of atherosclerosis;
    - o Quitting smoking should be a priority, using an interdisciplinary approach, involving family doctors, psychologists, cardiologists, pulmonologists, vascular surgeons;
    - o Implementation of vascular age calculation software in family medicine offices for the purpose of better communication with the patient.

Original contributions:

- In the present study, the progression of lesions of the arteries of the lower limbs was evaluated in a group of 106 patients, using duplex ultrasound and validated by angio tomography, and the evolution towards hemodynamically significant arterial stenoses was observed in 32.7% of the patients (**chapter 7.3, figure 7.1**);
- Thus, in the present study it was demonstrated that some of the classic cardiovascular risk factors were involved in the progression of peripheral arterial disease: smoking, dyslipidemia, diabetes, carotid atherosclerosis (**chapter 7.3, table 7.4, figure 7.6, figure 7.20, figure 7.8, figure 7.22**);
- In the multivariate analysis, the independent variables associated with the progression of peripheral arterial disease were: **the number of pack-years of smoking, the values of glycosylated hemoglobin and HDL-cholesterol and the presence of carotid atheromas** (**chapter 7.3, table 7.19**);
- In the present study it was shown that smoking significantly influenced the progression of PAD, but in logistic regression it did not have an independent

predictive value. Smoking intensity, measured by pack-years of smoking, has been shown to be a more accurate indicator for predicting PAD progression. On the other hand, among patients who quit smoking during follow-up, PAD progression was significantly lower (**chapter 7.4, paragraph 5**);

- Patient behavior was assessed, particularly smoking cessation. We showed that almost a third of patients managed to quit smoking after implementing our communication strategy, centered on the shared medical decision (**chapter 7.3, figure 7.2**);
- We used the concept of vascular age in the communication strategy. We estimated the vascular age by two methods, by cardiovascular risk factors and directly by measuring the intima-media thickness at the level of the carotid artery. In the present study, vascular age, calculated by both methods, was greater than biological age (**chapter 7.3, table 7.2**);
- Vascular age, as the age of the arteries, made the abstract concept of cardiovascular risk easier for patients to understand. Currently, preventive medicine must be based on a patient-centered approach, where therapeutic decisions are made in collaboration with the patient, who is empowered to participate in the management of his health condition. Explaining the abstract concept of cardiovascular risk to the patient is sometimes difficult. However, the idea of vascular age as the age of its arteries is easier for patients to understand, improving patient adherence to therapeutic decisions. It was tested to improve communication with patients, based on the implementation of the shared medical decision, by informing patients about premature vascular aging, using the concept of vascular age. Explaining the concept of vascular age had an additive effect on classical communication methods (**chapter 8.3. table 8.2**);
- The **utility of a lifestyle change intention questionnaire** was demonstrated, the results of which were directly related to smoking cessation. Patients who showed greater intention to change lifestyle following effective doctor-patient communication had a higher rate of smoking cessation (**chapter 8.3. table 8.1**);
- Following the complex analysis of cardiovascular risk factors, biological profile, comorbidities, subclinical atherosclerotic damage, lifestyle change adherence score, the **factors contributing to smoking cessation** were identified. We have shown that patients are influenced in their decisions rather by a parameter resulting from an

objective measurement, namely the vascular age calculated directly by measuring the intima-media thickness, compared to an abstract conversation related to cardiovascular risk factors (**chapter 8.3, table 8.3**);

- On multivariate analysis, the independent variables associated with smoking cessation were: **vascular age** estimated by intima-media thickness and lifestyle change **adherence score** (**chapter 8.3, table 8.29**);
- **Patient awareness** of the effects of smoking was also assessed, finding a poor perception of the link between smoking and peripheral arterial disease and of the coexistence of peripheral arterial disease with other cardiovascular events such as myocardial infarction and stroke (**chapter 8.3, figures 8.27 and 8.28**).

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