UNIVERSITY OF MEDICINE AND PHARMACY
„VICTOR BABEȘ" FROM TIMIȘOARA
FACULTY OF GENERAL MEDICINE
DEPARTAMENT VI CARDIOLOGY

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DOCTORAL THESIS

EVOLUTIVE CHARACTERISTICS AND PREDICTORS OF ARTERIAL DYSFUNCTION IN SPECIAL POPULATIONS (PREGNANCY): INSIGHTS AND IMPLICATIONS FOR HIGH BLOOD PRESSURE

PhD Supervisor

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T i m i ș o a r a
2022

INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality worldwide. In the European Union, more than 1,5 million people die yearly because of CVD, which means more than a third of the total deaths. Moreover, although in 2017, the life expectancy equalled 73 years, the healthy life expectancy was just 63 years, proving the existence of a decade of unhealthy status. Another finding of the Global Burden of Disease Study Report showed that whereas the communicable pathologies decreased by 41%, the non-communicable inflictions increased by 40%.

In recent years, the pattern of cardiovascular diseases has changed. In the past, atherosclerosis and its consequences were the prerogatives of developed countries; nowadays, this situation is also encountered in poor or developing countries. This shift of paradigm occurred due to social situation modifications such as the improvement of hygiene conditions, easy access to water and food, the prompt treatment of acute infections, and international support programs, which all led the population of these regions to live longer and, therefore, to suffer a more significant proportion of cardiovascular diseases. Another critical aspect of cardiovascular diseases is represented by disparities between countries, regions, races and genders. Although Romania is one of the places with the highest cardiovascular risk in Europe, the prevention programs are insufficient and most often, they do not include specific categories, e.g. pregnant patients and Roma minorities. In Romania, the death rate of CVD disease is more significant compared to the rest of the European Union countries (second place), with more than 60% of the total number of deaths, and another worrisome aspect is that in the rest of the developed countries, CV mortality decreased, in our country, it enhanced. Another discrepancy is gender-related. The data in the literature demonstrated that women are at higher risk of cardiovascular risk due to underdiagnosis and insufficient or late treatment. This situation is also maintained during pregnancy. Cardiovascular risk is not appropriately assessed, although CVD complicates around 4% of pregnancies and, in developed countries, represents the leading cause of death during gestation and postpartum. Furthermore, the foetus can suffer consequences in utero and long-term, having an increased risk of hypertension and chronic kidney disease, and the mother is also predisposed to high blood pressure, cardiovascular disease and stroke later in life

Recent years proved an exponential increase in cardiovascular disease among women due to advanced conception age, human reproduction techniques, smoking, obesity, sedentary lifestyle, western diet, stress, obesity, dyslipidaemia and diabetes. These risk factors are also responsible for increased arterial stiffness and early atherosclerosis, triggering the cardiovascular continuum and the cardiorenal metabolic syndrome. The most prevalent risk factor for CVD is high blood pressure. In the general population, according to the World Health Organization (WHO), 1.28 billion adults aged 30-79 years worldwide have high blood pressure, most (two-thirds) living in low- and middle-income countries and almost half are not aware of it, and so, untreated. Just 21% of adults with hypertension have it under control. Hypertension is also significantly encountered in pregnant women, affecting up to 10% of pregnancies, with an incidence of pre-eclampsia (PE) of about 3%. Because hypertension is a principal cause of premature death worldwide, one of the global targets for non-communicable diseases is to reduce the prevalence of hypertension by 33% between 2010 and 2030 and maybe, by identifying new, valuable, inexpensive, non-invasive, broadly applicable markers we might prevent or early diagnose and treat more effectively and achieve this goal. Hypertension and arterial stiffness are closely linked. Both lead to atherosclerosis and consecutive complications, and arterial stiffening may also increase irreversibly due to hypertension-induced structural changes in the artery because sustained blood pressure elevations accelerate atherosclerosis, arterial smooth muscle hyperplasia and hypertrophy, and collagen synthesis further increase arterial stiffness. Nonetheless, the process is incompletely discovered and understood, and as science evolves, novelty occurs, and the therapy must be adjusted. Because CVD is a fundamental cause of debilitating symptoms, inability to work, handicaps and tremendous healthcare costs, it is a problem for all of humanity. Among women, cardiovascular disease is the leading cause of death, and because underlying CVD risk factors are frequently present for years before the onset of the actual pathology, it is crucial to identify women who should undergo risk screening at a younger age through more innovative methods. Pregnancy and the postpartum period provide a window of opportunity to research the possibilities of early management due to certain pregnancy-related complications (hypertensive disorders of pregnancy, gestational diabetes mellitus, idiopathic preterm birth, delivery of a baby with intrauterine growth restriction, or placental abruption) that can help reliably identify women with masked CVD risk factors. Through the current paper, we intend to explore the implications of arterial stiffness and high blood pressure in several populations, focusing mainly on pregnant cohorts.

GENERAL PART

We aim to study the ability of specific markers of arterial stiffness to assess cardiovascular risk and forecast future cardiovascular events. In addition, the evaluation of arterial stiffness parameters employs non-invasive, inexpensive, reproducible, accessible, and painless techniques. We hope to demonstrate that they could be widely utilised in the screening of cardiovascular disease risk, subclinical phases, but also in the clinical stages, with clinical therapeutic implications and clear benefits for future outcomes. By identifying the risk factors that can cause arterial stiffness and studying the methods to reduce it, we can significantly reduce the prevalence of the cardiovascular disease. Cardiovascular risk factors can trigger a chain of events called “the cardiovascular disease continuum (CVDC)”. Left unattended can lead to end-stage heart failure and exitus. The main cardiovascular risk factors incriminated in CVDC are *high blood pressure (HBP)*, dyslipidaemia, obesity, diabetes mellitus (DM) and smoking. Cardiovascular risk factors will trigger alterations in the system and atherosclerosis with subsequent pathologies (coronary artery disease, myocardial infarction), left ventricular organic modifications (hypertrophy and dilatation), followed by dysfunction, end-stage heart failure, and end-stage heart failure death. A woman’s susceptibility to future vascular or metabolic disease may be revealed by the development of certain complications during pregnancy, and thus, we can consider it a veritable stress test. The cardiovascular risk assessment during pregnancy depends tremendously on the CV status and history of the mother. Suppose she has no history of CVD and no signs of it. In that case, she will only be evaluated briefly and at a basic level, meaning that if she is known with a history of CVD, she will benefit from a comprehensive history and physical examination, a 12-lead electrocardiogram, and transthoracic echocardiogram. These are often combined to generate risk scores, which are statistically derived. A systematic review and meta-analysis demonstrated that if a woman had in her history pre-eclampsia, that should be considered a risk factor because they observed that there was an increase in the relative risks (95% confidence intervals) for hypertension of 3.70 after 14.1 years of weighted mean follow-up, for ischaemic heart disease 2.16 after 11.7 years, stroke 1.81 after 10.4 years, and venous thromboembolism 1.79 after 4.7 years. In a *healthy pregnancy,* phenomena like increased insulin resistance, hyperlipidemia, hypercoagulability, inflammation, and hyperdynamic circulation occur. These are exacerbated in women with pre-eclampsia; some are also characteristics of metabolic syndrome, a cardiovascular disease "risk factor." A few CV RFs are associated with an enhanced risk of pre-eclampsia, such as insulin resistance, obesity, systemic inflammation, preexisting hypertension, diabetes mellitus, and chronic kidney disease. Pre-eclampsia may increase the risk of future cardiovascular disease due to a shared cause, or subclinical vascular damage occurs during pre-eclampsia. Moreover, pre-eclampsia is considered a type 5 cardiorenal syndrome. We emphasise that pre-eclampsia can be considered a veritable cardio-renal-metabolic syndrome due to the combination of metabolic syndrome and renal injury factors. It is widely accepted and demonstrated that ageing leads to vascular modifications and increased arterial stiffness. Moreover, vascular status can be used as an indicator of biological age versus chronological age. Apart from the irreversible and unstoppable ageing process, other risk factors considered "classic", such as hypertension, dyslipidaemia, diabetes, obesity, contribute to early vascular changes, starting from the smallest to the most significant vessels. There are various invasive and non-invasive ways to assess arterial function. The most utilised and preferable modalities include carotid-femoral pulse wave velocity (cf-PWV), brachial-ankle PWV (ba-PWV), Cardio Ankle Vascular Index (CAVI), pulse wave analysis (PWV) and augmentation index (Aix). All parameters rely on the velocity of the pulse wave throughout the arterial tree, but the gold standard recommended in the guidelines is the cf-PWV. CfPWV predicts cardiovascular events and all-cause mortality in patients with hypertension, chronic kidney disease, the elderly, and the general population.

SPECIAL PART – PERSONAL CONTRIBUTIONS

Our teamwork includes three separate studies interconnected by the central phenomenon: hypertension. Our research aimed at answering the following questions:

a) Does arterial function modify during gestation compared to non-pregnancy status, and if so, is it different according to the pregnancy trimester?

b) Can arterial stiffness evaluation and the values of its parameters predict the occurrence of hypertension and future events in pregnant women?

c) Do lifestyle measures, e.g. physical training, influence arterial function and if so, in what way?

Study number one - Assessment of the arterial function in healthy pregnant women vs. non-pregnant women

Arterial stiffness is positively associated with hypertension, stroke, heart failure, and ischemic artery disease, with an increase in the risk of a first major cardiovascular event, which is the main cause of death in pregnant women in developed countries. In addition, the arterial stiffness is related to the foetus and neonatal outcomes as well. Arterial function parameters could be useful tools to detect early modifications in the vascular function of pregnant patients before clinical manifestations and further complications occur in both mother and foetus. They could also serve for the assessment of therapeutic results. The lack of reference values in pregnant patients limits their applicability in clinical practice. The current study aimed to compare arterial stiffness in pregnant and non-pregnant women and assess the differences. There are no reference values for gestational period and previous research was based on numbers for the general population. In all pregnant patients with pregnancy-related diseases, chronic conditions prior to gestation, or risk factors, or even healthy women, it is necessary to assess their arterial stiffness parameters as predictors for possible future events. It is mandatory to thoroughly study the arterial stiffness before, during and after gestation on large groups of subjects and to establish the exact cut-off values for this population. This prospective study was performed in patients admitted to the Obstetrics and Gynaecology Department of “Pius Brînzeu” Emergency Clinical County Hospital of Timisoara, Romania, during a 10-year period (between 2010–2019). The study was performed with the approval of the Ethics Committee of the hospital mentioned above and it was conducted according to the established principles of the Declaration of Helsinki. First, 1000 patients were included in the study. Patients with high blood pressure, cardiac arrhythmias, diabetes, obesity, hypercholesterolemia, chronic kidney disease, and systemic vasculitis, were excluded from the study. The final group consisted of 887 pregnant and non-pregnant healthy women, aged between 20–45 years, after they signed the informed consent.The patients were divided into two different groups: group 1 - healthy primiparous pregnant patients (N1 = 471, 53.1%), without comorbidities or risk factors and group 2 - control, healthy non-pregnant patients (N2 = 416, 46.9%).

Personal data about lifestyle, smoking status, exercise, medical history (including family history), anthropometric features (such as age, height, weight, body mass index (BMI)) and results of arterial function assessments were collected. In group 1 (healthy pregnant), data about arterial functions were collected during four separate visits, in the first, second and third trimester of pregnancy, and 6 weeks post-delivery. In addition, data about the gestational period, the foetus weight and gender and delivery type (vaginal or caesarean) were added.

The pulse wave was analysed through an easy, non-invasive method and an accredited medical device: Arteriograph (TensioMed, Hungary). Each patient was prepared 24 h prior to the test, and the procedure was explained (according to the “patient info” document). For PWV determination, the protocol from the technical book of the device was followed. The complex arterial function assessment included parameters such as systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), heart rate (HR), aortic SBP (SBPao), aortic pulse wave velocity (PWVao), and augmentation index (AIx). All measurements were performed with the same device, TensioMed Arteriograph, mentioned before. In pregnant women, brachial AIx decreased progressively during pregnancy and increased again in post-partum (the mean value in the first trimester was −47.41% ± SD, in the second trimester it was −51.93% ± SD, and in the third it was −58.40% ± SD; in post-partum, it increased to −45.57% ± SD). PWVAo started to decline in the second trimester and continued to decline in the third trimester; in post-partum, it was increased compared to the first trimester (mean PWVAo in the first trimester was 6.78 m/s ± SD, in the second trimester 5.99 m/s ± SD and in the last trimester 5.93 m/s ± SD; in post-partum it increased to 7.17 m/s ± SD). In order to see if there are significant differences in the four tested time points, the ANOVA unifactorial test and Friedman test were applied, resulting in extremely significant differences ($p < 0.001$) between the brachial AIx (%) and PWVao values during the study period. Brachial AIx % decreased until the third trimester, when it started to increase again until the post-partum stage. PWVao decreased in the second trimester, it was maintained in the last trimester, and increased again in post-partum, even more, compared to baseline (first trimester). The following were the mean values for the gestational period (39.34 weeks ± SD), APGAR score (9.22 ± SD) and foetus weight (3468.97 g ± SD). To see if physical activity can modify the outcomes, both the Student *t* unpaired test and Mann–Whitney test were applied, for each group separately. Significant differences were obtained ($p < 0.05$) for BMI, brachial AIx, PWVao, SBP, DBP, PP, SBPao and HR values. In group 1, women who regularly exercised (33.97%) were compared to women who did not exercise (66.03%). We also compared them in all 4-time moments: first, second, third trimester, and in post-partum. Patients who are physically active had statistically significant better outcomes ($p < 0.05$) for delivery, anthropometric features, hemodynamic status and arterial functions.

Reference values for pregnant women need to be established for better assessment and early detection. In the current study, healthy pregnant women had statistically significantly different values compared to healthy non-pregnant women; thus, larger prospective studies are necessary, to establish reference values for arterial function parameters for this category.

Considering the literature data, we hypothesised that pregnant women would have a different pattern of arterial function compared to non-pregnant women. This field is yet to be explored. Some studies concluded that in physiological pregnancy, there is a significant decrease in unadjusted aortic PWV from pre-conception to the second trimester. In the present research an interesting progression of the arterial markers was observed: starting from baseline (at enrolment), brachial AIx% decreased until the third trimester, and then started to increase again until the post-partum stage. PWVao decreased in the second trimester, and was maintained in the last trimester, and increased again in post-partum, even more compared to baseline (first trimester). This specific pattern should be studied in larger cohorts in the future.

Nonetheless, further studies are necessary to confirm whether PWV reduction by this approach can directly prevent CVD in pregnant women. It is necessary to work with appropriately adjusted parameters alongside raw data. There is little information in the medical literature regarding the effects of therapy on arterial function. This study offers insights regarding PWV analysis and additional information about arterial stiffness in pregnant women versus conventional brachial blood pressure measurements. In addition, the arterial pattern in pregnant women is different from non-pregnant women. This could be a potential base for further research regarding the importance of PWV study for the assessment, management and prediction of pregnancy-related cardiovascular events. Future perspectives include establishing exact cut-off values of arterial stiffness for healthy pregnant women and demonstrating the objective benefits of physical therapy during pregnancy in larger groups. The results obtained in this study proved that arterial function is modified during pregnancy and these alterations differ according to the trimester of gestation; there was a decrease of both Aix brachial and PWV in the second and third trimester, followed by a post-partum increase. In addition, women who regularly exercised had ameliorated anthropometric features, hemodynamic status and delivery outcomes. Due to these specific changes that occur in normal healthy pregnancies, further studies are necessary to establish cut-off values for this category of patients, in order to detect early changes in arterial stiffness parameters. Arterial stiffness assessment represents a useful tool in the early diagnosis of pregnancies at risk and can lead to better routine care and better outcomes for these patients, through early detection and physical activity.

Study number two - Assessment of the arterial function in hypertensive pregnant women

This research was intended to analyse the arterial stiffness in three categories of patients, hypertensive pregnant women vs. healthy women (both pregnant and non-pregnant). The clinical implications of this research consist in lowering the burden of both target organ damage and clinical CVD through arterial stiffness decrease, preventing further complications secondary to pregnancy-induced hypertension (PIH) for both mother and foetus. To achieve these goals, we need to accurately and early assess and diagnose arterial function before high BP develops. The results obtained may open a door for future studies and could offer the possibility of implementing new, non-invasive, inexpensive strategies and guidelines for early detection, diagnosis and treatment of PIH.

The case-control study was performed between 2018 and 2019 in the Obstetrics and Gynaecology Department of “Pius Brînzeu” Emergency Clinical Hospital, Timisoara, Romania; more than 4000 patients were consecutively enrolled. The criteria for subjects’ selection were as follows: inclusion criteria - women aged 20–40 years-old, with or without pregnancy. For the pregnancy groups, an ultrasound scan was routinely performed to confirm the gestational age; maternal char- acteristics and medical history were recorded. For the gestational groups, as inclusion criteria were considered also the normotensive singleton pregnancy and the absence of major foetal defects or aneuploidy. All the women included were healthy and ordinarily active, and they were not under pharmacological treatments; exclusion criteria - age <20 or over 40 years-old, the presence of major chronic diseases such as pre-existing diabetes, CVD, chronic hypertension (HTN), nephropathies or impaired renal function (serum creatinine higher than 1.0 mg/dL), autoimmune diseases, malignant conditions, thrombophilia, previous pregnancy with gestational HTN, PE, or other adverse perinatal outcomes. Also, there were excluded pregnancies ending in foetal death or miscarriage before 24 weeks of gestation or termination of pregnancy and cases of incomplete evaluations. All measurements were routinely performed. The pregnant patients were followed-up regularly. Non-pregnant women were assessed just once because they were healthy and did not present any condition or risk factor for potentially significant modifications of the cardiovascular parameters (the control group). Pregnant women were assessed monthly, but a complex assessment with arterial stiffness parameters evaluation was performed four-times: at inclusion in the study (first trimester), in the second and third trimester, and postpartum. The database was created using the Microsoft Excel program. The patients were assigned into three groups, as follows: in the group 1 there were included healthy pregnant women at enrolment and who only developed BP >140/90 mm Hg (HTN) after 20 weeks of gestation (hypertensive pregnant patients, N1 50, 33.3%); group 2 comprised healthy pregnant patients (N2 50, 33.3%), without comorbidities at enrolment and who remained healthy throughout the pregnancy; in the group 3 (control group) there were assigned healthy, non- pregnant patients (N3 = 50, meaning 33.3%). From the beginning, we aimed to have equally divided groups, and the statistical expert stated that at least 33 patients are necessary for a significant study. Because hypertensive patients were the fewest, the number of patients in the other groups was adjusted accordingly. Simple randomisation leads most of the time to unequal group sizes; stratified block randomisation was used in order to control and balance the in- fluence of covariates and that is why all groups had equal sizes. For all the patients, there were collected information about age, height, weight, BMI, the brachial AIx, PWVao (m/s) values, SBP, DBP, PP (brachial pulse pressure in mm Hg, which is the difference between systolic and diastolic BP), SBPao and HR values. The patients were questioned about their smoking status and physical activity. For groups 1 and 2, there were created additional fields: the brachial AIx, PWVao values in dynamics, for the first, second, and third pregnancy semesters, and 6 weeks postpartum.

The entire research study was conducted according to the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects); also, it was approved by the Ethics Committee of the “Pius Brînzeu” Emergency Clinical County Hospital of Timisoara, Timis County, Romania. Each patient included in the study signed the informed consent form.

Arterial stiffness was assessed by Medexpert Arteriograph (TensioMed, Hungary) device. According to the device specifications, the following parameters were assessed: SBP, DBP, HR, and PP. Also, AIx was followed, being calculated as the difference between the am- plitudes of the late (backward) systolic wave (P2) and the early (for- ward) systolic wave (P1) over the pulse pressure (PP) and multiplied with 100; Aix = (P2-P1) / PP *100; aortic AIx – central augmentation index (%) calculated based on a very strong ($R > 0.9$) linear relationship. between the brachial and central augmentation index; ED – ejection duration of the left ventricle (ms), it is the period of the mechanical systole, i.e. the time-span between the opening and closing of the aortic valves. PWVao was calculated from the travelled distance (measured as the suprasternal notch – pubic bone distance) of the pulse wave in the aorta divided by the measured transit time (RT/2). For PWVao, the reference values begin at 6.1 (4.6–7.5) m/s for young, healthy individuals. There are no reference values for pregnant women. Hypertensive disorders of pregnancy are classified into mild HTN (SBP 140–159 mm Hg and/or DBP 90–109 mm Hg) or severe HTN (BP 160/110 mm Hg). This classification takes into consideration the timing of the first diagnosis of HTN and the persistence of high BP after delivery. The next categories are described in the recent European guidelines: pre-existing HTN: HTN diagnosis before pregnancy, early in preg- nancy (before 20 weeks of gestation), or HTN continues six weeks postpartum; gestational HTN: HTN first diagnosis during pregnancy, after 20 weeks of gestation; it usually resolves within six weeks postpartum. It is considered a form of secondary HTN; pre-existing HTN plus superimposed gestational HTN with proteinuria; PE; antenatal unclassifiable hypertension. The PE term is used when HTN is first diagnosed after 20 weeks of gestation, and it is unclear if HTN was pre-existing. Reassessments six weeks postpartum will help distinguish pre-existing from gestational HTN. Currently, the diagnosis of PE endorsed by the International Society for the Study of Hypertension in Pregnancy (ISSHP) includes new-onset HTN (systolic >140 mm Hg and diastolic >90 mm Hg) along with one or more other features: proteinuria, other maternal organ dysfunction (including liver, kidney, neurological), or haematological involvement, and/or uteroplacental dysfunction, such as foetal growth restriction and/or abnormal Doppler ultrasound findings of uteroplacental blood flow.

The mean age was approximately 30 years in all three groups, and the BMI was 26.2 kg/m2 in the first group, 20.1 kg/m2 in the healthy pregnant women and 21.13 kg/m2 in the healthy non-pregnant patients. No obese patients were included in the study. In the first trimester, there were no differences between the groups regarding BP and PP. The highest BP values were met in the HTN pregnant group, with a mean of 157.52/102.86 mm Hg, and the lowest in the healthy pregnant subjects, with a mean of 106.24/61.62 mmHg, whereas the control group ranged in the middle. PP values were the highest in the first group and the lowest in the second group. At the enrolment moment, there were no differences between the three groups. In the second trimester, the highest BP values were in the HTN pregnant group.

The central SBP and HR tendencies, showed increased values in the HTN pregnant group and the lowest values in control patients, with a mean of 166.58 mm Hg for BP and 80.76 bpm for HR in the first group vs 104.66 mm Hg and 71.74 bpm. Brachial AIx was calculated in all three trimesters of pregnancy and postpartum for groups 1 and 2, and only at enrolment moment for group 3.Mean PWVao in group 1, in the first trimester, was 7.74 m/s ±SD; in the second trimester it was 7.75 m/s±SD and in the last trimester 8.80 m/s ±SD. Postpartum, it decreased slightly to 8.37 m/s±SD. For the second group, PWV was lower compared to the group one throughout the whole pregnancy; it had a decreasing tendency during gestation, with a slight increase postpartum. The mean PWV in group 3 was 7.41± SD m/s. Regarding BP values, group 1 included only hypertensive patients, 34% having severe HTN and 23.5% of them developing PE, 66% had mild high BP. None of the patients with severe HTN performed any physical activity, and 41.2% smoked. Almost half (42.4%) of the pa- tients with mild HTN had physical activity, and 63.6% smoked. None of them developed PE. In group 2, 68% performed physical activity, 44% smoked, and none developed PE. In group 3, more than half (52%) performed physical activity and smoked (54%). Histograms were depicted to see the distribution of systolic and diastolic BP in the three groups; the reference values were the same for all groups included in the study (BP < 120/80 mm Hg). In order to see the data distribution, the Kolmogorov-Smirnov normality test was applied and resulted that data of this study were not normally distributed ($p > \alpha = 0.05$); thus, nonparametric tests were used. The Mann–Whitney test was applied to see if there were differ- ences between group 1 and group 2, respectively between group 2 and group 3, regarding age, BMI, the brachial AIx, PWVao values, SBP, DBP, PP, SBPao and HR values. For the first Mann-Whitney test ran between group 1 and 2, there were obtained significant differences (p < 0.001) for most of the tested variables (SBP, DBP, SBPao, PP, brachial AIx, PWV, BMI), except HR (significant differences (p 0.006) were ob- tained) and the age variable (no significant differences (p 0.694 > 0.05)). For the second Mann-Whitney test, ran between groups 2 and 3, resulted as follows: significant differences (p < 0.001) for the SBP, DBP, and the PWVao values; significant differences (p < α, α 0.05) for BMI, SBPao (p 0.02), brachial AIx [%] (p 0.01) and PP (p=0.049). No significant differences (p > α, α 0.05) resulted for age (p=0.939) and HR (p=0.384). A multilinear regression model was applied to see if there is an as- sociation between BMI and BP (both systolic and diastolic). In group 1 resulted a positive strong significant correlation (p < 0.001, r=0.835). In the 2nd group, it was obtained a weak significant positive correlation (p

= 0.042, r = 0.288) and in the third one, a weak positive insignificant correlation (p 0.677, r 0.128). As well, it was applied a linear regression model in order to see if there is an association between BMI and PP. In group 1 resulted a positive medium significant correlation ($p < 0.001$, r=0.582). In the 2nd group, it was revealed a weak significant positive correlation (p 0.014, r 0.211) and in the last group, a weak positive insignificant correlation (p 0.467, r 0.105). Thus, it was emphasized that a higher BMI is associated with elevated BP in pregnant women, and the association is more pronounced in hypertensive pregnant women. In the current research, there were observed significant ($p < 0.001$) differences for the hemodynamic and arterial stiffness parameters and significant differences (p=0.006) for the heart rate (HR) between pregnant women, with or without HTN.

Women with PIH had different values of the arterial function parameters long-time before the first signs of high BP occurred. Also, BMI had a deleterious effect in all patients, but especially in this category. This was consistent with the previous studies, where women with higher early pregnancy BMI had increased SBP and DBP than their normal-weight counterparts throughout pregnancy. Also, there were obtained significant differences ($p < 0.001$) for BP and aortic PWVao, and significant differences ($p < 0.05$) for BMI, aortic systolic BP (p=0.02), brachial AIx (p=0.01) and PP (p=0.049) values between groups 2 and 3. Arterial stiffness increases with age and BP; these are the major determinants of PWV and linearly related to BPs and, symmetrically, at any level BP is dependent on the quadratic age and PWV increases by 1.5-fold between the younger/lower BP and the elderly/higher BP. The association of age with various arterial distensibility parameters has been exhaustively described in the literature and proved that PWV varies with age and gender. The average adult values of PWV are 7.4 m/s for females and 8.2 m/s for males. The reference values are presented per age decade and BP category. These normal and reference values represent a critical step in the implementation of PWV as a clinical tool for detecting subclinical organ damage in the routine patient workup. Unfortunately, there are no reference values for pregnant women. Previous studies showed that oscillometry-determined AIx, SBPao and PWVao have similar values to the invasively-obtained ones. Arterial stiffness, measured as aortic PWV, and wave reflection, measured as AIx, are independent predictors for total and cardiovascular morbidity and mortality. In the current study, the arteriography was used to assess arterial stiffness of the patients with extra cautions to cuff placement and steadiness of the patient, because the most significant limitations of this technique are the positioning of the cuff and the necessity for a complete motionless arm. It was observed that compared to the variance and reproducibility of the PWV measurements, the arteriography had the least variation. Moreover, it was demonstrated that atherosclerosis detection and assessment of arterial stiffness is paramount, because they are associated with cardiovascular outcomes, independently of traditional risk factors (such as ageing, HTN, diabetes, dyslipidaemia, obesity, and smoking). Women in particular pose a greater risk regarding appropriate medical conduct. They remain insufficiently diagnosed and subsequently undertreated or mistreated. The main reasons for this situation are atypical symptoms, late presentation in the doctor's office and the general belief that oestrogen offers protection before menopause and thus, the risk is underrated. Another important aspect in the female population is represented by obesity, which is more frequently encountered in women, and subsequently, a greater risk for diabetes mellitus. Diabetes can be both a risk factor and a consequence of CVD. Risk factors like dyslipidaemia, high BP, high BMI, and fatty liver disease are often encountered in patients with diabetes, making them vulnerable for cardiovascular morbidity. Pregnant women are a special category, because they cannot be treated for diabetes, high BP or dyslipidaemia as standard patients, due to the contraindications of the treatment. Therefore, it is mandatory to carefully assess the balance risk/benefit of the drugs and most importantly, to prevent any stage that could lead to the necessity of medical treatment. To achieve that, physicians need to rely on any technique, test or device that can predict or improve the early diagnosis in pregnant women. In this spirit, PWV measurement is a simple and reliable non-invasive tool to improve detection and risk stratification for CVD. Unadjusted aortic PWV decreases significantly from preconception to the second trimester in normal pregnancies. That is consistent with the results obtained, and this trend was maintained in the third trimester as well and increased in postpartum, showing a progression in pregnant women with physiological gestation compared to non-pregnant patients. That was concordant with the results from previous studies. There were not observed the same modifications in the hypertensive pregnancies. The less known and proved aspect of this correlation is that the values are higher in pregnant women predisposed to HTN, which can therefore be considered a useful predictor for very early diagnosis and treatment. Compared to similar published data, the current study offered an additional comparison (to the group of healthy non-pregnant women) to the standard analogy of the arterial stiffness in healthy vs unhealthy pregnant women. This three-arm study was our option in order to observe all modifications that occur in the arterial tree of women, both in an out of pregnancy, both with and without HTN. Moreover, the reason for this comparison was represented by the lack of clear thresholds for pregnant women. Another novelty that this paper brings to the field consists of measuring multiple parameters for arterial stiffness, not only the PWV. Additionally, we quantified Aix brachial and PP for a more comprehensive approach. One of the main findings of this study shows the arterial stiffness alterations that naturally occur during pregnancy, and it was consistent with the results from the literature. Data obtained add further information and emphasize the necessity of large, randomised trials for establishing reference values for pregnant women, to assess their cardiovascular risk accurately. Furthermore, cut-off values need to be established according to the gestational period, because we proved that it modifies from one trimester to another and from pregnancy to post- partum, which is consistent with other studies. Another finding of the study was that women who develop HTN during pregnancy had increased arterial stiffness compared to women who remained healthy throughout the pregnancy. The results obtained bring in the possibility of a new, standard, non-invasive, low-cost and fast measurement that can be used both as a predictor and a prognostic factor in pregnant women. The consequences of predicting the risk of pregnancy-induced HTN and PE would lead to increased focus on these patients, more frequent follow-ups and acknowledging the patients of the risk and controlling all the other existing risk factors (such as obesity, sedentary lifestyle, stress, smoking, salt excess, sleep apnoea). If all non-pharmacological measures fail and HTN develops, it is paramount to diagnose early and treat immediately, thus decreasing the risk of complications for both mother and foetus. This research may be an exclamation mark and an open door for future studies and, hopefully, the beginning of large trials where all these results and conclusions can be reproduced. That could lead to guideline modifications and different approaches in clinical practice. It is needed to apply this in hypertensive pregnant women as well, not only in the general population. However, an accurate assessment needs a reference values interval specific for pregnant women. The limit of the study and difficulty of applying the current results resides in the relatively small size of the groups. In the future, we consider expanding the scale and add follow-ups with measurements at least one year after the 6 weeks postpartum visit. The current study demonstrated that the arterial function changes throughout the pregnancy period in both healthy and hypertensive women enhance the arterial stiffness, which is correlated to the degree of BP increase. It emphasizes the importance of arterial function parameters and BMI as markers for future BP values and outcomes throughout gestation and the necessity for physicians to inform and take firm actions to acknowledge the risk factors and to lower women's risk of complications. Advanced maternal age and other risk factors are endangering women during gestation and expose both mother and foetus. Because the leading cause of maternal death during gestation is CVD, it is important to detect cardiovascular problems before they become clinically manifest. Arterial stiffness assessment is a useful tool that can detect even the slightest modification in the arterial function before the appearance of clinical signs.

Study number three - The effects of physical training on arterial function in hypertensive patients

Physical training has several benefits on the heart and blood vessels but the precise, intrinsic mechanisms of the body adjustments to exercise are less clear. Current clinical guidelines have extensive recommendations concerning lifestyle changes for patients with hypertension accompanied by sustained and regular physical training. Based on the previous controversial data, we aimed to study the impact of personalized, specific physical training and lifestyle changes on selected peripheral and central hemodynamic parameters and also on arterial stiffness.

A total of 129 hypertensive patients were included. They were selected according to the following criteria: controlled BP values of up to 140/90 mm Hg, stable medication for at least 30 days before the beginning of the study, age between 40 to 70 years, and good communication skill of the patient with the doctor. We established as exclusion criteria any condition non compatible with physical training, uncontrolled blood pressure, other cardiovascular disease such as ischemic heart disease, significant valvular pathology (greater than grade II), cardiomyopathy, congenital heart disease, myocarditis, pericarditis, and lack of cooperation. All these patients were treated and were instructed according to the ESC/ESH guidelines regarding hypertensive patients. After inclusion, we divided the patients into two groups: group A=63 patients, who gave their informed consent to be involved in a physical training program, and group B with 66 patients who refused to participate in the physical training program but gave their consent to be enrolled in the present study as part of the control group. We assessed the hemodynamic parameters and arterial stiffness at baseline and at 4 months in both groups. Patients in group A performed an exercise test at baseline in order to evaluate the exercise capacity and to include them in the rehabilitation program.

For parameter evaluation, we used an arteriograph device (TensioMed, Budapest, Hungary). The system is equipped with software able to measure several hemodynamic parameters and arterial stiffness. The vascular parameters we aimed to assess in this study were: SBP, DBP, PP, aortic systolic bood pressure (SBPao), aortic pulse pressure (PPao), aortic pulse wave velocity (PWVao). These parameters were evaluated at the beginning of the study and at 4 months.

The patients had 15 to 20 min total rest before initial evaluation of vascular parameters in a quiet room with normal temperature. Alcohol or coffee consumption and smoking were forbidden 12 h, and 3 h respectively before evaluation. Parameters were registered before breakfast in supine posture. Two assessments were recorded every 5 min, and the final value represented the mean of the two measurements for each group.

Physical training design. The intervention was applied only to group A, and consisted of 50 min of exercise training, four times a week for 4 months, under the supervision of a licensed physical therapist highly experienced in cardiovascular rehabilitation. The exercise training program comprised a combination of 30 min of aerobic exercises and 20 min of resistance exercises per session. Aerobic exercise training was performed on an ergometer bike and treadmill. The exercise sessions was also performed at an intensity of 60% of each patient's age-predicted maximal heart rate reserve. In order to supervise the exercise intensity and energy expenditure of the patients, we used Polar FT80 heart rate monitors (manufactured by Polar Electro, Kempele, Finland).

Resistance training used the methods previously described by Ho et al. (8) and consisted of 2-3 sets, 10 repeated procedures per set at 10-repetition maximum (RM) of various resistance exercises: leg press, leg curl, leg extension, bench press, and rear deltoid row. Each major muscle group involved in resistance exercises was evaluated weekly using the 10RM test: patients performed each exercise at a selected weight and if they completed fewer than 8 or more than 12 repetitions, the weight was adjusted accordingly and the exercise re-attempted after resting until the 10-RM level was determined. Each patient was evaluated at the baseline and at the end of the study protocol (after 4 months) with regard to arterial stiffness and physical performance. For each patient, physical training was stopped when the assessed parameters such as BP and heart rate reached 80% of their values evaluated during the preliminary exercise test.

Ethical considerations. Informed consent was signed by all patients enrolled in this study before medical procedures started. The Ethics Committee of Victor Babeş University of Medicine and Pharmacy Timişoara (Romania) approved research methodology and all procedures were performed according to the World Medical Association Declaration of Helsinki.

Statistical analysis used SPSS software, version 17 (SPSS Inc, Chicago, IL, USA); a p-value less than 0.05 was considered statistically significant. The physical exercise was individualized taking into consideration hemodynamic parameters, age and tested exercise capacity at baseline in order to obtain maximal benefit. At the baseline of the study, the differences between the two groups were not statistically significant.

Significant differences were registered between the two groups after 4 months of physical training combined with usual medication administered to the patients before and during our study. As general parameters, the patients enrolled in group A improved their life quality by changing their lifestyle, which had a strong impact on their work and personal life. Patients from group A became more active and declared that they did not become tired as quickly as they had before the enrollment in the present study. Patients from group A declared that during the 4 months of the study, the physical training had become a habit. They also stated that their exercise capacity had gradually increased. They also registered an enhancement of self-confidence and will to live. In contrast, group B patients became more sedentary and with a decreased effort capacity. In group A, there was also a tendency of wanting to increase the intensity of the treatment quickly, but for safety reasons, this was tempered by the therapist.

The improvement of the hemodynamic parameters was significant in involved patients undergoing physical training. We compared the parameters before and after 4 months of exercise training supervised by the specialized physical therapist.. For this group, we only made recommendations on lifestyle change.

By evaluation of the seven parameters included in the present study, significant differences were observed between the trained group A compared with control group. PWV decreased for 60 patients in group A (n=63) and only for 14 patients from group B (n=66). The most important changes were for the PWV (Figure 1), SBPao (Figure 2) and PPao (Figure 3) in group A. Most changes in group B were not statistically significant, except PPao which increased. DBP and DBPao at baseline and at 4 months did not differ between the two groups. During the study, five patients needed to stop the exercise session due to high BP during effort (>200 mmHg).

The best results translated as a significant improvement of the arterial stiffness (PWV) and SBPao, both with great clinical importance in vascular aging and secondary complications to hypertension (which are more likely to be caused by SBPao than brachial SBP) were registered in group A. Our results supported a negative impact of a lack of physical training for group B, where these parameters had tendency to deteriorate over time.

We emphasize that parameter differences between groups are highly representative for further studies in this direction and for the elaboration of new recommendations regarding lifestyle changes in general and physical training, especially in hypertensive patients (cardiovascular rehabilitation program).

The general clinical benefits of physical training applied for patients with high BP are well recognized and accepted today. Our results demonstrate that changing the lifestyle is not enough to reduce cardiovascular risk, but we must elaborate adequate and individualized rehabilitation programs evaluated by a panel of hemodynamic parameters comparatively assessed. We need to maximize the benefit of physical exercise and to improve peripheral and central hemodynamic parameters and arterial stiffness. It was shown that by enhancing these parameters, we can reduce cardiovascular morbidity and mortality. Although group B also received recommendations according to the ESC prevention guidelines, the parameters were not improved compared to those from group A trained with the rigorous rehabilitation program. By physical activity in general, and physical training in particular, we can improve both hemodynamic and stiffness parameters in order to make a model for delaying arterial aging. We believe that the is great benefit to be gained from rehabilitation programs for hypertensive patients due to the cost–benefit ratio in favor of public health. Based on our results, we believe that exercise training programs improve hemodynamic parameters such as SBP, PP, SBPao, and PPao. Thus, physical training as part of cardiovascular rehabilitation has a positive impact on arterial stiffness parameters such as PWVao. Rehabilitation programs are a safe and effective method for reducing cardiovascular risk. Exercise training may delay stiffening of the large arteries (aorta) and in this way perhaps delay arterial ageing.