CLINICAL INVESTIGATION OF ARTERIAL STIFFNESS PARAMETERS WITH A NOVEL OSCILLOMETRIC DEVICE

Ph.D. Thesis Summary

by

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1. INTRODUCTION

A large body of evidence is available on the crucial role of preclinical organ damage in determining cardiovascular (CV) risk in individuals. Arterial stiffness parameters are commonly used for this purpose with the aim to identify structural and functional changes in the arteries in the development of atherosclerotic disease.
The independent predictive value of aortic stiffness (aortic pulse wave velocity – PWVao, aortic augmentation index – AIXao) has been demonstrated for fatal stroke, all-cause and CV mortalities, fatal and nonfatal coronary events in hypertensive, diabetic, endstage renal disease, in elderly patients and in the general population. The examination of the above-mentioned parameters, however, has not become part of the daily routine in clinical work so far.
New techniques allow us to investigate regional aortic stiffness and local carotid stiffness simultaneously. Carotid stiffness may be of particular interest because in that artery, atherosclerosis is frequent, especially in patients with verified coronary artery disease (CAD).
Type 2 diabetes mellitus is also known to carry a high CV risk. Therefore, the assessment of PWV as a target organ damage marker should be an important part of ambulatory risk stratification in patients with coronary artery disease and those with type 2 diabetes mellitus (T2DM).

2. OBJECTIVES

The overall goal of our study was to prove the reliability of the new oscillometric device, the Arteriograph, in the detection of preclinical arterial damage by measuring arterial stiffness noninvasively.

2.1. Invasive validation of the Arteriograph

The aim of our study was the complex and invasive validation of the hemodynamic parameters (SBP, AIX, PWV) measured by the Arteriograph on patients who underwent routine coronary angiography.

2.2. Comparison of aortic and carotid arterial stiffness parameters in patients with verified coronary artery disease

We aimed to compare regional aortic stiffness and local carotid stiffness parameters measured by two different non-invasive methods in patients with verified coronary artery disease. Further, we compared and contrasted non-invasive stiffness parameters to the coronary SYNTAX Score in patients who had undergone coronary angiography. All measurements were done simultaneously using carotis color Doppler echo-tracking system (Aloka SSD-5500, Tokyo, Japan) and oscillometric occlusive equipment (Arteriograph, TensioMed, Budapest, Hungary).

2.3. Comparison of arterial stiffness parameters in patients with coronary artery disease and diabetes mellitus using Arteriograph

We aimed to compare arterial stiffness parameters (PWVao and AIXao) between two high cardiovascular risk groups: patients with verified coronary artery disease (CAD) or with T2DM, using the Arteriograph device. We also aimed to determine the cut-off values for PWVao, AIXao; and to calculate the sensitivity and specificity of arterial stiffness parameters in verified CAD and T2DM.

3. THE STUDY POPULATION

The invasive validation of Arteriograph was performed on patients who underwent routine coronary angiography.
For the examination of arterial stiffness parameters with different non-invasive methods and in different patient population, a total of 186 CAD, 152 patients with T2DM and 186 apparently healthy, medication-free, asymptomatic control subjects were investigated aged between 40 and 84 years. Exclusion criteria were arrhythmia, valvular heart disorders and heart failure {New York Heart Association (NYHA) criteria III-IV}.
The protocol of this clinical study was reviewed and approved by the local Institutional Ethics Committee. Written informed consent was obtained from all patients who participated in the study. The investigation conforms to the principles outlined in the Declaration of Helsinki.
In the CAD group, coronary artery disease was verified by coronary angiography. All patients underwent routine coronary angiography (using the Judkins technique) on digitised coronary angiography equipment (Integris, Philips). Coronary angiograms were computerised and assessed by 3 experienced angiographers who were blinded to the results of arterial stiffness measurements. For this study, we defined significant CAD as showing at least 50 % or greater stenosis, or at least 75 % or greater flow-reduction in one coronary artery. Patients in the CAD group received appropriate medical treatment (angiotensin-converting enzyme inhibitor, angiotensin II receptor blocker, statins, low-dose aspirin, beta-blockers) according to the relevant guidelines.
Patients in the T2DM group were free from known coronary artery disease and were treated with oral anti-diabetic and other (angiotensin-converting enzyme inhibitor, angiotensin II receptor blocker, calcium channel blocker, statins, aspirin) drugs. Diabetes was diagnosed by hemoglobin A1C level ≥6.5 % and fasting plasma glucose ≥7.0 mmol/l, or abnormal oral glucose tolerance test (OGTT level after a 2 hour interval is equal or more than 11.1 mmol/l) or a previous diagnosis of T2DM. The antidiabetic treatment was monitored with the measurement of serum hemoglobin A1C level.
Age- and gender-matched control subjects were randomly selected from a previously collected database of apparently healthy, medication-free, asymptomatic subjects.
Smoking status was defined as current or past use of cigarettes.

4. INVASIVE VALIDATION OF THE ARTERIOGRAPH

4.1. Methods

4.1.1. Invasive measurements

Our work was carried out in the Hemodynamic Laboratory of the Heart Institute of PTE (University of Pecs, Medical School, Hungary) and in the Hemodynamic Laboratory of University of Rome ‘La Sapienza’, Polo Pontino, (Italy).
Standard (5 French), fluid-filled, pigtail catheters were used to record early and late systolic pulse pressure wave signals.

4.1.1.1. Intra-aortic (AIx-ao) versus Arteriograph-measured brachial (AIx-br) augmentation index

In 16 cases, we measured the Aix-ao with an intra-aortic cannula positioned into the aortic root and the Aix-br with Arteriograph simultaneously on identical heart cycles.

Altogether 154 identical pulse waves were compared in the range of the AIx-ao from -13.0 to 58.9%.

4.1.1.2. Invasively measured versus Arteriograph-calculated central systolic blood pressure

In 55 cases, simultaneous invasive (in the aortic root) and noninvasive measurements were performed to compare the central SBPao values obtained by these two different methods. The Arteriograph calculates the central SBPao on the basis of the brachial SBP and the pulse pressure curve, measured together in the same process on the upper arm.

4.1.1.3. Comparison of the invasively measured true aortic pulse wave velocity between the aortic root and bifurcation and the Arteriograph-measured aortic pulse wave velocity

In 22 cases, the invasively and noninvasively measured PWVao values were compared. In 13 cases, the PWVao was determined with one catheter by pulling it back from the aortic root to the bifurcation under X-ray control, and the transit time of the pulse wave was measured using ECG gating. In nine cases, we used two catheters (inserted from radial and femoral artery) positioned to the aortic root and to the aortic bifurcation. The transit time of the pulse wave between these two points was measured simultaneously on identical heart cycles.

4.1.2. Statistical analysis

First, descriptive statistics were calculated for both the invasively and noninvasively (Arteriograph) measured parameters (Table 1).

Variable	AIX (n=10)	PWV (n=22)	SBPao (n=55)
Age (years)	56 ± 10	62 ± 8	66 ± 9
Men, n (%)	8 (50)	12 (55)	43 (74)
Weight (kg)	82 ± 14	82 ± 13	78 ± 14
Height (cm)	170 ± 10	169 ± 9	168 ± 7
SBP (mmHg)	150 ± 27	152 ± 25	154 ± 24
DBP (mmHg)	88 ± 15	87 ± 14	93 ± 12
Hypertensive,[a] n (%)	10 (63)	16 (73)	43 (74)
HR (beats/min)	77 ± 14	68 ± 11	71 ± 12
PP	62 ± 16	65 ± 17	61 ± 15

Table 1. Participants characteristics and descriptive characteristics.

AIX: aortic augmentation index; DBP: diastolic blood pressure; HR: heart rate; PP: pulse pressure; PWV: pulse wave velocity; SBP: systolic blood pressure; values are mean ± SD. [a]If SBP is over 140 mmHg.

Bland–Altman analysis was performed to assess the comparability of the two methods (differences were calculated as invasive value – noninvasive value). Linear regression analysis was also carried out to define the relationship and correlation coefficients between the invasive and noninvasive variables. Continuous variables are indicated as mean and standard deviation (SD), and categorical variables as percentages. A probability of less than 5% (two-tailed) was taken as indicative of statistical significance.

4.2. Results

4.2.1. Comparison of intra-aortic (Aix-ao) and Arteriograph measured brachial (Aix-br) augmentation index

A strong, linear and significant correlation was found between the invasively recorded aortic and Arteriograph- measured brachial Aix on both identical beat to beat and average Aix per patient basis, with R=0.9 (P<0.001) and R=0.94 (P<0.001), respectively (Fig. 1A and B). Equally strong correlations (R=0.9 for beat to beat and 0.95 for mean value per patient) were found if automatic, second-derivative-based determination was used to assess the invasively measured aortic Aix. The range of the aortic Aix varied between -13.0 and 58.9% in the studied group, which practically covers a wide range of the possible values. The observed very strong linear correlation between Aix-ao and Aix-br, especially in the averaged Aix values/patient group, allowed us to calculate the aortic Aix from the brachial Aix in the Arteriograph software using the regression equation y=0.5062x+37.636. By using this formula the invasively recorded and Arteriograph-calculated aortic Aix had become comparable with Bland–Altman plot, because of the same dimensions. Bland–Altman comparisons showed acceptable accuracy; that is, more than 95% of the differences were within +2SD and the mean differences between the methods were only 0.0% (mean values per patients) and -0.2% (beat to beat). The limits of agreement for the beat to-beat comparison were 11.6% (mean+2SD) and -12.1% (mean-2SD) (Fig. 1C and D).

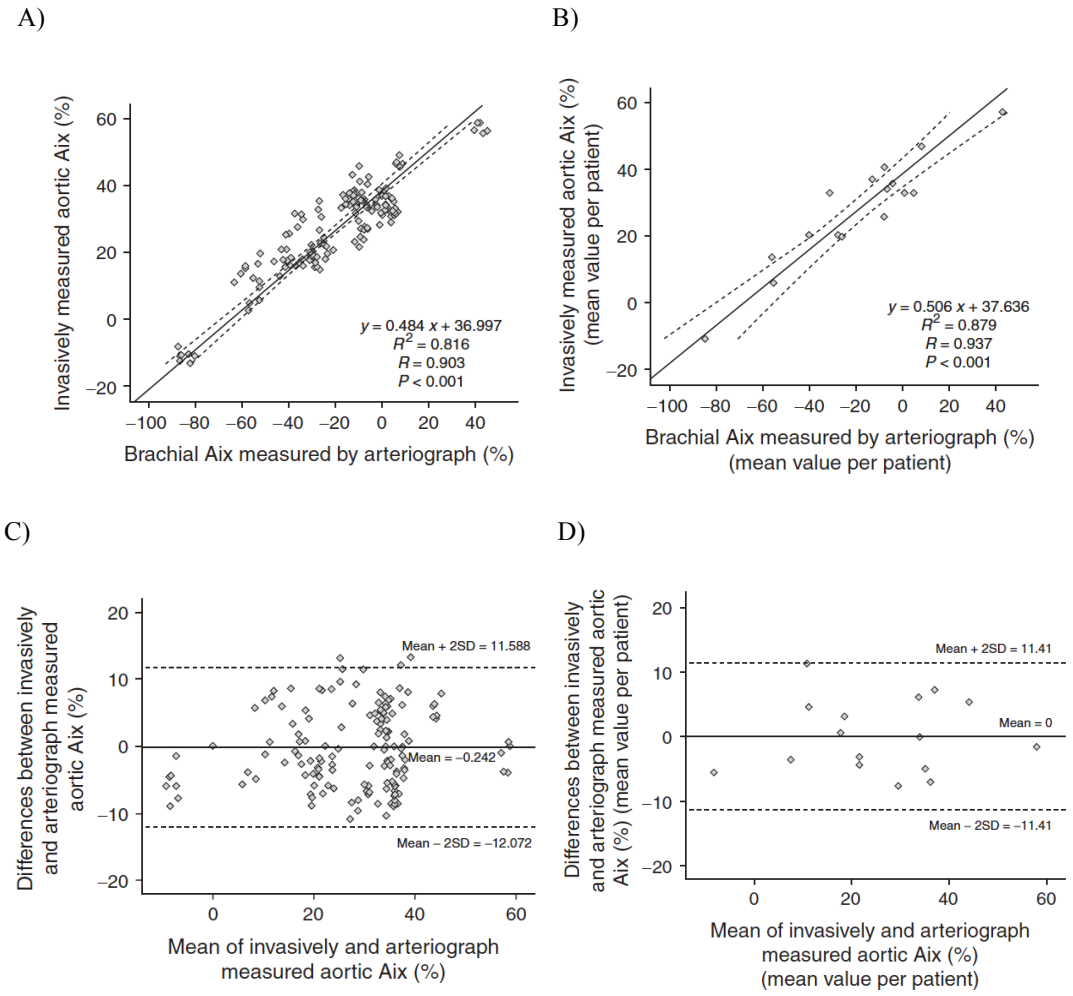

Figure 1. Comparison of intra-aortic and Arteriograph-measured brachial augmentation index.
A) Relationship between brachial Aix measured by Arteriograph and invasively measured aortic Aix (beat-to-beat basis). B) Relationship between brachial Aix measured by Arteriograph and invasively measured aortic Aix (mean value per patient). C) Bland–Altman analysis of mean values and differences for aortic Aix measured invasively and by Arteriograph (beat-to-beat basis). D) Bland–Altman analysis of mean values and differences for aortic Aix measured invasively and by Arteriograph (mean value per patient).
Aix: aortic augmentation index.

4.2.2. Invasively measured versus Arteriograph-calculated central systolic BP

Very strong and significant correlation (R=0.95; P<0.001) was found between the invasively measured and the Arteriograph-calculated SBPao (Fig. 2A). The mean SBPao of the 55 patients was 158.1 (±26.4) mmHg for the invasive and 158.6 (±26.9) mmHg for the oscillometric measurements with no significant difference found between them (P= 0.63). As shown by the Bland–Altman plot (Fig. 2B), more than 90% of the paired readings were inside the 2SD range and the mean difference was merely 0.56 mmHg between the methods. The limits of agreement were about ±17mmHg; however, 91% of the paired comparisons were within 15mmHg, 82% within 10 mmHg and 60% within 5mmHg of differences (Fig. 2C).

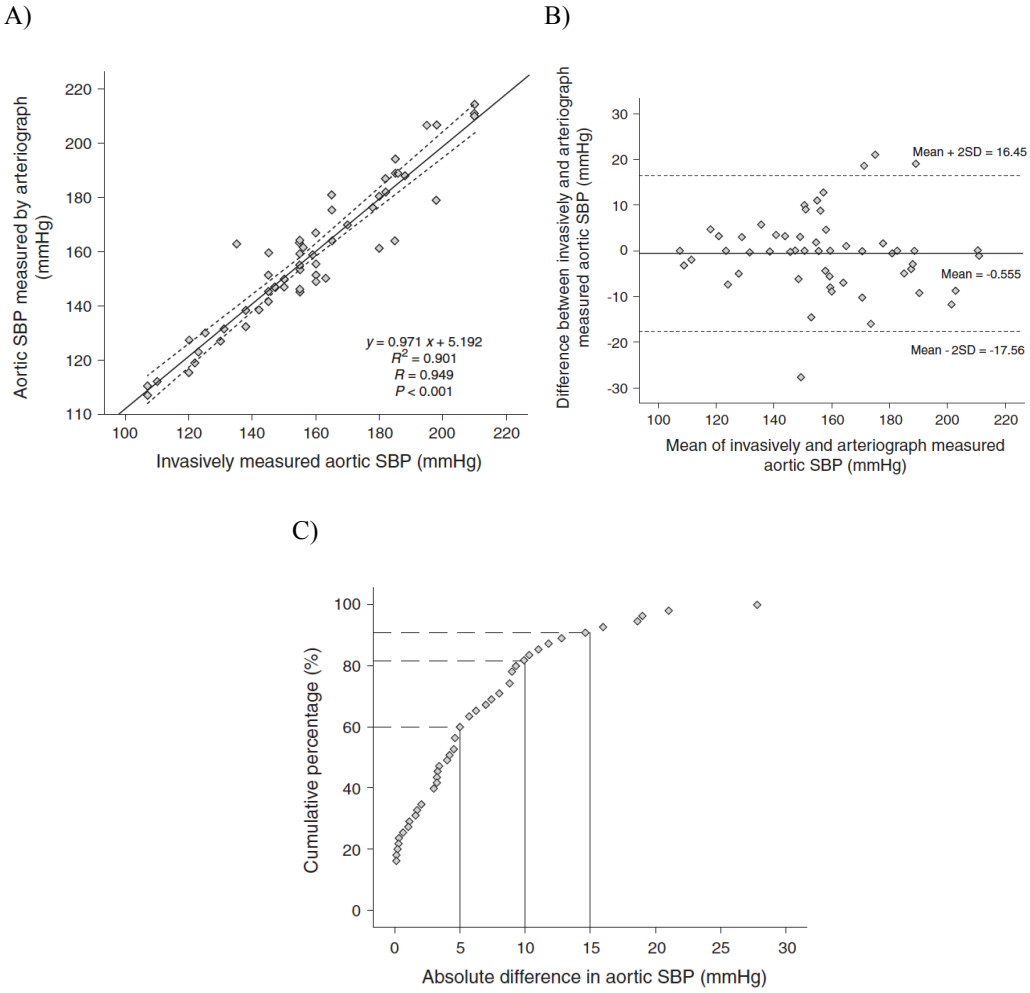

Figure 2. Invasively measured aortic SBP versus central SBP calculated by the Arteriograph device.

A) Relationship between invasively measured and Arteriograph-calculated aortic SBP. B) Bland–Altman analysis of mean values and differences. C) Cumulative percentage of absolute difference in central aortic systolic pressure. British Hypertension Society criteria level 'B'.
SBP: systolic blood pressure.

4.2.3. Comparison of the aortic pulse wave velocity measured invasively and with oscillometric Arteriograph device

The mean of the PWVao values measured invasively versus Arteriograph was 9.41 ± 1.8 m/s and 9.46 ± 1.8 m/s, respectively, and the difference between the PWVao values was not significant (P=0.77). The Pearson's correlation coefficient between the invasively and noninvasively measured PWVao proved to be R=0.91 (P< 0.001) (Fig. 3A). Using the Bland–Altman plot (Fig. 3B) most of the differences (90.9%) were within the mean ± 2SD range and the limits of agreement were 1.49 and -1.59 m/s. There was no systematic trend in the differences between the two methods that is the accuracy was approximately the same across the whole PWVao range. Accordingly, regression analysis yielded not significant results (P=0.83, the slope of the regression line did not differ significantly from 0).

A) B)

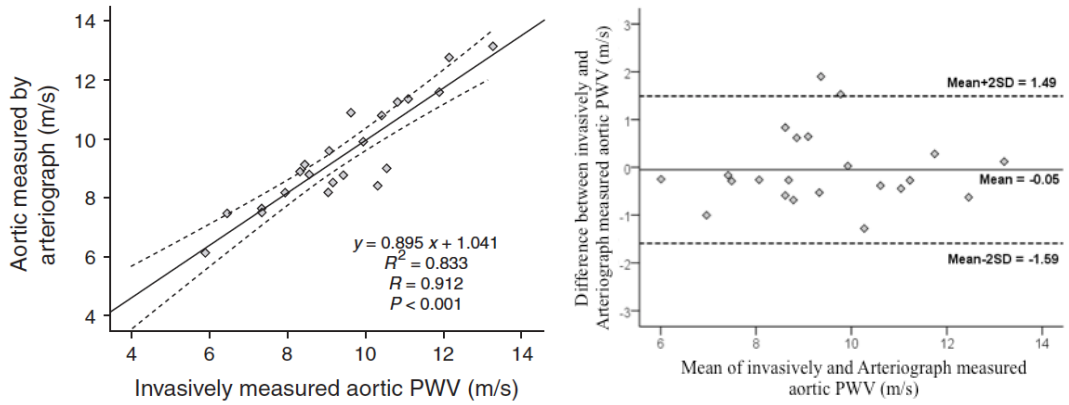

Figure 3. Comparison of the aortic pulse wave velocity between the aortic root and bifurcation, measured invasively with intra-aortic catheter and with oscillometric Arteriograph device.

A) Relationship between invasively measured aortic PWV (mean of manually and automatically obtained values) and aortic PWV measured by Arteriograph. B) Bland–Altman analysis of mean values and differences.

PWV: pulse wave velocity.

4.3. Discussion

The most important result of our study is the strong, significant correlation between the invasively and oscillometrically measured (calculated) aortic Aix, central SBP and aortic PWV values.

The observed strong linear correlation between the invasively measured Aix-ao and the Aix-br measured by Arteriograph on the brachial artery allows the determination of the central (aortic) Aix with a satisfactory level of accuracy for the clinical routine, without using the special mathematical method, the highly controversial generalized transfer function (GTF).

Despite the strong correlation, discovered during the comparison of the central (aortic) and the noninvasively measured SBP value, using the Bland–Altman plot the 2SD range turned out to be approximately ±17 mmHg wide. The Arteriograph reached British Hypertension Society grade B, which is considered acceptable for clinical use.

Another reason why the oscillometric, occlusive technique provided more accurate results than the radial applanation tonometry for assessing SBPao, may be the fundamental difference in the measuring methods. During applanation tonometry the flow is maintained throughout the entire cardiac cycle and in the compressed artery, thus the shape of the pulse curve is influenced by the Bernoulli effect. However, Arteriograph utilizes the occlusive method (suprasystolic pressure, stop flow in the brachial artery): there is no flow in the artery at all, and consequently pulse pressure waves can be recorded without the influence of the Bernoulli effect.

In the present study, we found significant correlation between the invasively measured true aortic PWV and the oscillometrically determined PWVao with limits of agreement acceptable for clinical practice. These results are better than the correlations found in earlier studies where PWVao values, determined by the

Arteriograph, were compared with noninvasively measured carotid–femoral PWVs recorded by applanation tonometry (SphygmoCor) and by a piezoelectric (Complior) device.

One of the main reasons for the high level of conformity between the PWVao measured by Arteriograph and the invasively obtained measurements, may be the fact that by this method we could find values closer to the true aortic PWV than with c-f PWV measurement because the time interval between two systolic pulse peaks during brachial stop-flow condition reflects the time difference between the direct and the reflected aortic waves; consequently, only the aorta is represented as a vessel during the measurement. In the case of the c-f PWV determination, the transit time is influenced by arteries with different PWVs, such as carotid, iliac and femoral arteries. Furthermore, the opposite direction of wave propagation in the case of Complior and the beat-to-beat variability of the isovolumetric contraction time during ECG gating for sequential measurement by SphygmoCor may also decrease the accuracy of measuring true PWVao. The above-mentioned circumstances might result in the fact that the PWV variance and repeatability measured by c-f PWV method – considered to be the 'gold standard' so far – turned out to be much worse compared with Arteriograph.

The association between c-f PWV and the aortic PWV has only been discussed by few publications so far. The article of Weber et al. compared the invasively measured aortic PWV to the c-f PWV measured by the SphygmoCor device but the measurements were not performed at the same time. In another study, a more favorable setup was used concerning the accuracy and comparability as invasive and c-f PWV (Complior) measurements were performed simultaneously in a group of patients with coronary artery stenosis (CAS) as well as in CAS-negative participants. The overall Spearman's correlation was 0.7, which was similar to the findings of the previous study, although a significant difference was observed between the two groups (CAS positive, R=0.74; CAS negative, R=0.46). Based on these findings, the authors concluded that the invasively measured aortic PWV and the c-f PWV should not be used interchangeably.

The surprisingly high agreement between the oscillometrically and the invasively measured PWVao may provide data for answering the several decades old question about the reflection site of the aortic pulse wave. The unique setup in our study using two aortic catheters positioned into the root and to the bifurcation allowed us to measure identical heart cycles and to eliminate completely the errors caused by the varying isovolumetric contraction time during ECG-gated sequential pulse wave recording.

The observed strong correlation ($R=0.9$; $P<0.001$) for the nine patients, examined in this arrangement, proves that the propagation time from the aortic root (arch) to the bifurcation and the time between the peaks of direct and reflected waves, recorded by Arteriograph, are basically identical. According to these findings we can conclude that the forward wave is reflected with a high probability from the area of the bifurcation.

5. COMPARISON OF AORTIC AND CAROTID ARTERIAL STIFFNESS PARAMETERS IN PATIENTS WITH VERIFIED CORONARY ARTERY DISEASE

5.1. Methods

5.1.1. Patients

We studied 125 CAD patients (mean age 62±10 years) and 125 age-and gender-matched, apparently healthy, control subjects.

All measurements were done simultaneously using carotis color Doppler echo-tracking system (Aloka SSD-5500, Tokyo, Japan) and oscillometric occlusive equipment (Arteriograph, TensioMed, Budapest, Hungary).

5.1.2. Carotid stiffness (Echo-tracking)

The Aloka Color Doppler system with a 7.5 MHz linear array probe, and an echo-tracking subsystem were used for recording the wave intensity data. The data were updated with a frequency of 1 kHz and the steering angle of the ultrasound beam never exceeded ± 20° for any recording. Blood pressure was simultaneously measured with a cuff-type manometer applied to the upper arm as a required input to the Aloka system. The maximal and minimal values of changes in diameter of the artery were calibrated by systolic and diastolic blood pressure. The wave intensity was averaged over a minimum of four heartbeats at the same site in the artery. The diameter and wall motion of the right common carotid artery were measured 2 cm below the carotid bifurcation. This Doppler echo-tracking system allows the determination of local PWVcar and AIxcar data using on-line one-point measurements.

5.1.3. Diagnosis of CAD and calculation of the SYNTAX Score

The SYNTAX Score was calculated with an interactive question-based computer program. The algorithm consists of twelve main questions referring to the coronary anatomy, total number and extent of coronary artery lesions. In our study, the SYNTAX Score was calculated for each coronary lesion producing a ≥ 50% luminal obstruction in vessels with diameter 1.5 mm or over. Patients were randomised according to 2-year MACE (major adverse coronary events) rates to low (0-22), intermediate (23-32), and high (≥ 33) SYNTAX Score groups. In our study 64 patients were in low, 18 in intermediate and 43 patients in high Syntax score groups. After scoring these individual lesions, the total SYNTAX Score was determined and correlated to regional and local arterial stiffness parameters.

5.1.4. Statistical analysis

The values were expressed as mean ± SD. We used simple regression analysis to evaluate linear association between aortic and carotid stiffness parameters. The correlation coefficient was defined as r according to Spearman. Differences between control subjects and subjects with CAD were tested with the 2-tailed *t* test. A $p<0.05$ was taken as the level of statistical significance.

5.2. Results

The characteristics of the CAD patients and control subjects are summarised in Table 2.

Variable	Control group (n=125)	CAD group (n=125)	p-value
Age (years)	62±10	62±10	
Male, n (%)	97 (78)	97 (78)	
Weight (kg)	82.1±15.3	84.4±15.2	0.020
Height (cm)	171±9	170±8	0.379
BMI (kg/m^2)	28.2±4.4	29.3±4.3	<0.01
SBP (mmHg)	139±18	135±21	0.056
DBP (mmHg)	83±10	80±14	0.034
MAP (mmHg)	101±12	98±15	0.029
HR (beat/min)	73±12	70±12	<0.01

Table 2. Characteristics of the patients with verified coronary artery disease (CAD group) and healthy control subjects (control group).

Data are presented as mean ± SD.

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; HR: heart rate.

Fig. 4A illustrates the results of regional PWVao, which was measured by the occlusive oscillometric method. We found a significant increase in regional PWVao for the CAD patients compared to the control subjects (10.1±2.3 m/s vs. 9.6±1.5 m/s; p=0.019). Similarly, significant differences were observed between the two groups when AIxao values were compared (34.2±14.6% vs. 30.9±12% for the CAD and control groups, p = 0.05; see Fig. 4B).

A) B)

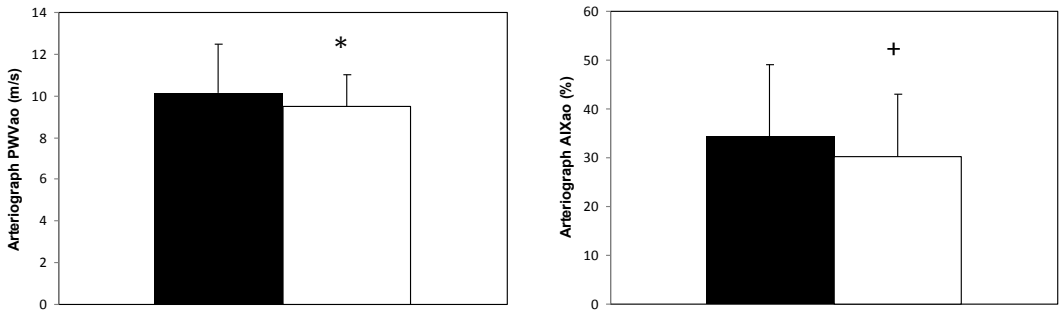

Figure 4.

A) Comparison of regional (aortic) pulse wave velocity (PWVao) in patients with verified CAD (CAD group) with age- and gender-matched apparently healthy control subjects (control group). **B)** Comparison of regional (aortic) augmentation index (AIx) in patients with verified CAD (CAD group) with age- and gender-matched apparently healthy control subjects (control group).
These measurements were carried out with occlusive, oscillometric device (Arteriograph).

= CAD group (n= 125)
= control group (n= 125)

Data are presented as mean ± SD. * = $p<0.05$; + = $p=0.05$

In 35 of the 125 CAD patients, simultaneous measurements were taken by the carotid echo-tracking method to determine local arterial stiffness parameters. As shown in Fig. 5A, we found a significant increase of the local PWVcar for the CAD patients compared to the control subjects (7.4±1.3 m/s vs. 6.5±1.1 m/s; $p<0.01$). Further, the CAD patients exhibited elevated AIxcar values compared to the control group. (19.4±10.7% vs. 5.1±9.8% for CAD and control group, $p<0.01$; Fig. 5B).

A) B)

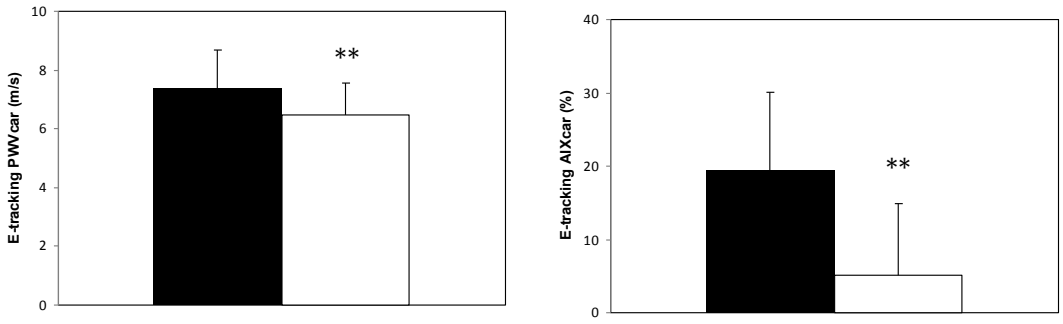

Figure 5.

A) Comparison of local (carotid) pulse wave velocity (PWVcar) in patients with verified CAD (CAD group) with age- and gender-matched apparently healthy control subjects (control group). **B)** Comparison of local (carotid) augmentation index (AIx) in patients with verified CAD (CAD group) with age and gender-matched apparently healthy control subjects (control group).
These measurements were carried out with Doppler echo-tracking method.

= CAD group (n= 35)

= control group (n= 35)

Data are presented as mean ± SD. ** = $p<0.01$

Fig. 6. shows the correlation between regional (aortic) and local (carotid) arterial stiffness parameters in patients with verified CAD. As PWV regards, we found a significant positive correlation between PWVao values that were measured by Arteriograph, and PWVcar values which were determined by echo-tracking method ($r=0.57$, $p<0.001$; Fig. 6A). Similar correlations were observed between regional (AIxao) and local (AIxcar) augmentation index values which are plotted in Fig. 6B ($r=0.65$, $p<0.001$).

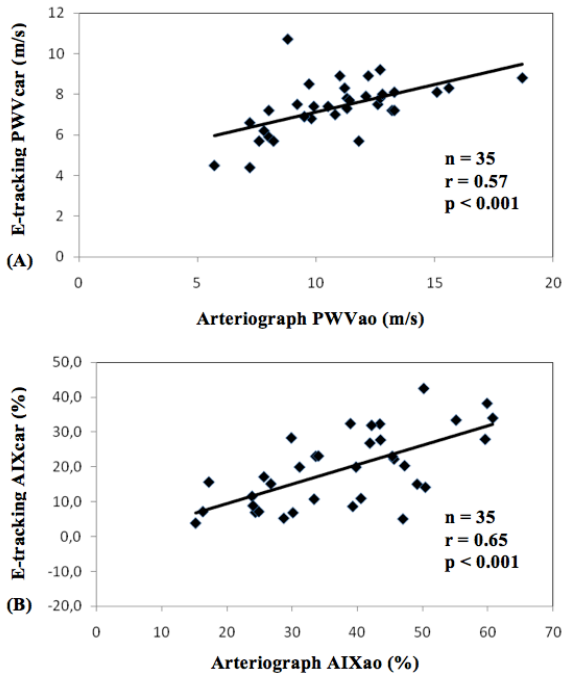

Figure 6.

A) Relation between regional (aortic) and local (carotid) PWV parameters in patients with verified CAD. The aortic pulse wave velocity (PWV_{ao}) was measured by the occlusive, oscillometric device (Arteriograph). The carotid pulse wave velocity (PWV_{car}) was determined by the Doppler echo-tracking (e-tracking) method. **B)** Relation between regional (aortic) and local (carotid) AIx parameters in patients with verified CAD. The aortic augmentation index (AIxao) was measured by the occlusive, oscillometric device (Arteriograph). The carotid augmentation index (AIxcar) was determined by the Doppler echo-tracking (e-tracking) method.

When correlating the Syntax Score and regional arterial stiffness parameters we did not find any significant correlation. Similarly, the coronary SYNTAX Score did not correlate with the carotid stiffness parameters (PWVcar and AIxcar; data not shown).

5.3. Discussion

In this study we first aimed to evaluate the correlation between regional (aortic) and local (carotid) arterial stiffness in patients with verified CAD. We also contrasted arterial stiffness parameters which were obtained by two different non-invasive methods to the coronary SYNTAX Score in patients who had undergone coronary angiography.

The principal contribution of the present study is that we found a significant increase of both aortic and carotid PWV and AIx values determined by occlusive oscillometric device and carotid echo-tracking method in patients with verified CAD compared to healthy counterparts. These observations have clearly indicated that PWVao and PWVcar provide similar information on impaired arterial stiffening in CAD patients. A further important observation of our clinical study is that there is a strong correlation between aortic and carotid stiffness parameters measured by two different non-invasive methods.

In our study we demonstrated that in patients with clinical organ damage AIx significantly increased, clearly indicating an impaired arterial function. These results are in good correspondence with earlier findings: central AIx proved to be an

independent predictor of mortality in hypertension, in end-stage renal disease, and in patients undergoing percutaneous coronary intervention. Our measurements also indicate a strong correlation of AIx data simultaneously conducted through oscillometric and echo-tracking techniques. There are numerous articles in the literature which can prove the relationship between carotid intima-media thickness and pulse wave velocity in patients with different type of atherosclerosis. In our study, we applied Doppler echo-tracking system to determine local carotid PWVcar and AIxcar. This novel method provides valuable data about local arterial stiffness that are different from those parameters that we can obtain from the intima–media thickness (IMT) measurements.

Although the local carotid and central aortic stiffness parameters correlated significantly, they are not identical for several reasons. Concerning the pulse wave velocity the interchangeability does not stand at all, because both vessels are basically different in their characteristics. However, not only morphological differences cause the lack of interchangeability between local (carotid) and regional stiffness. Until today, only the aortic PWV proved to be independent predictor of hard outcomes.

In contrast, we did not find any significant correlation when local and systemic arterial stiffness parameters were compared to the coronary SNTAX Score in patients who underwent coronary angiography. From this observation we concluded that although the increase of local and regional stiffness parameters correlated significantly with the impaired arterial function in patients with CAD, unfortunately stiffness parameters can not provide any information about the arterial damage in the coronary vessels. Furthermore, arterial stiffness represents the function of the inner lining of the endothelium and therefore it is very hard to correlate to the severity and extent of the coronary heart disease.

6. COMPARISON OF ARTERIAL STIFFNESS PARAMETERS IN PATIENTS WITH CORONARY ARTERY DISEASE AND DIABETES MELLITUS USING ARTERIOGRAPH

6.1. Methods

6.1.1. Patients

We investigated 186 CAD patients (61±9 years, age range: 40-84 years) and 186 age- and gender-, mean blood pressure and heart rate-matched control subjects, randomly selected from a previously collected database of apparently healthy, medication-free, asymptomatic subjects.

We evaluated 152 patients with T2DM (61±9 years; age range: 40-82 years), while 152 age- and gender-, mean blood pressure and heart rate matched subjects comprised the control group, randomly selected from the previously mentioned large database.

5.1.2. Statistical analysis

Continuous data are reported as mean ± SD. The clinical parameters of the matched populations were compared by using the Student's paired t-test, with the significance level set at 0.05. Multiple regression analysis was performed to investigate the relationship between arterial stiffness indices, clinical parameters, and the use of antihypertensive, diabetes, antilipid medications. Discrimination was calculated with

the areas under the receiver-operating characteristic (ROC) curves in case of CAD, T2DM and control subjects for both PWVao and AIXao. An area of 1.0 would indicate perfect discrimination, while 0.5 means the absence of discriminatory power.

6.2. Results

Demographic, clinical, haemodynamic and medication characteristics are summarized in Table 3.

Variable	Control group (n=186)	CAD group (n=186)	p-value	T2DM group (n=152)	p-value
Age (years)	61±9	61±9		61±9	
Male, n (%)	138 (74)	138 (74)		112 (74)	
Weight (kg)	81±15	84±15	0.050	88±16	0.020
Height (cm)	171±9	170±8	0.379	171±9	0.870
BMI (kg/m^2)	27.6±4.1	29.12±4.28	<0.05	30±4.5	<0.05
Smokers, n (%)	12 (7)	39 (21)	0.001	18 (12)	0.010
SBP (mmHg)	136.7±17.0	136.7±21.2	0.940	136.8±17.4	0.930
DBP (mmHg)	81.3±10.1	81.2±13.1	0.910	81.4±11.5	0.920
MAP (mmHg)	99.8±11.5	99.7±15.4	0.940	99.9±12.0	0.930
HR (beat/min)	69.2±11.4	69.1±12.4	0.900	69.3±10.8	0.940
Hypertension (%)	0	59	<0.001	44	<0.001
Glucose (mmol/l)	5.3 (4.3-5.9)	5.6 (4.2-6.3)	0.390	6.9 (3.7-9.9)	<0.001
HbA1c (%)				7.1±1.5	
Creatinin (µmol/l)	68.3±16.5	69.3±17.5	0.077	73.8±19.5	0.035
eGFR (ml/min)	92.3±21.5	89.3±20.5	0.067	85.9±24.5	0.020
TC (mmol/l)	5.4±0.9	5.6±1.2	0.202	5.7±0.8	0.123
HDL-C (mmol/l)	1.5±0.3	1.4±0.4	0.306	1.3±0.3	0.050
LDL-C (mmol/l)	3.3±0.4	3.5±0.5	0.060	3.6±0.8	0.020
Triglyceride (mmol/l)	1.3 (0.7-1.8)	1.3 (0.8-1.9)	0.522	1.6 (0.6-2.7)	0.009
Treatment					
BB (%)	0	76	<0.001	48	<0.001
ACEI/ARB (%)	0	74	<0.001	51	<0.001
ASA (%)	0	80	<0.001	19	<0.001
Statins (%)	0	75	<0.001	33	<0.001
CCB (%)	0	34	<0.001	13	<0.001
Nitrate (%)	0	40	<0.001	4	<0.005
Oral antidiabetics (%)	0	0		68	

Table 3. Descriptive statistics of healthy control subjects, patients with known coronary artery disease (CAD), and with type 2 diabetes mellitus (T2DM)

Data are presented as mean ± SD or median, p values for control subjects.

SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; HR: heart rate; eGFR: estimated glomerular filtration rate; TC: total cholesterol; HDL-C: high-densitiy lipoprotein cholesterol; LDL-C: low-densitiy lipoprotein cholesterol; BB: beta blocker; ACEI: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; CCB: calcium channel blocker

When we compared the CAD group to the age-, gender-, mean blood pressure-, and heart rate-matched, apparently healthy control group we found that PWVao and AIXao values in CAD patients were significantly higher (Table 4). In the T2DM population PWVao was significantly higher compared to the control group, whilst no significant differences were seen in the AIXao. We made comparison with the age-, gender-, mean blood pressure-, and heart rate-matched CAD and T2DM groups, and found non-significant differences in PWVao (p=0.10) and markedly lower AIXao in the T2DM group (p<0.001) (Table 4).

	Control group (n=186)	CAD group (n=186)	p-value	T2DM group (n=152)	p-value
PWVao (m/s)	9.3±1.5	10.2±2.3	<0.001	9.7±1.7	<0.05
AIXao (%)	31.9±12.8	34.9±14.6	<0.05	29.3±13.0	0.10

Table 4. Indices of arterial stiffness in patients with coronary artery disease (CAD), type 2 diabetes mellitus (T2DM) and healthy control subjects.

Data are presented as mean ± SD.

The impact of antihypertensive, antilipid, oral antidiabetic medications (ACEI/ARB, beta-blockers, calcium channel antagonists, nitrates, statins, sulfonylureas and metformin) on measures of arterial stiffness was also investigated in our study population. In multiple regression analysis the use of ACEI/ARB was the only significant determinant of the stiffness parameters (Table 5).

Variable	PWVao (r)	PWVao (p)	AIXao (r)	AIXao (p)
Age	0.39	<0.001	0.26	<0.001
Heart rate	0.21	<0.001	-0.35	<0.001
SBP	0.41	<0.001	0.10	0.35
ACEI/ARB	-0.16	0.03	-0.13	0.04

Table 5. Multiple regression analysis of PWVao and AIXao.

Correlation coefficients of multiple regression (r) and the level of significance are only shown when p<0.05. SBP: systolic blood pressure; ACEI/ARB: angiotensin converting enzyme inhibitor/angiotensin receptor blocker.

Statistics explored a cut-off value of 10.2 m/s for PWVao and 33.2% for AIXao in the comparison of CAD and healthy control subjects with acceptable area under curve (AUC), sensitivity and specificity data (Table 6).

	CAD group				T2DM group	
Variable	**PWVao (m/s) ***		**AIXao (%) ****		**PWVao (m/s) *****	
	Value	**95 % CI**	**Value**	**95 % CI**	**Value**	**95 % CI**
AUC	0.61	0.54-0.67	0.57	0.51-62	0.57	0.52-0.61
Sensitivity	0.66	0.55-0.72	0.58	0.50-0.66	0.62	0.52-0.7
Specificity	0.57	0.51-0.66	0.58	0.52-0.68	0.55	0.51-0.61
Positive predictive value	0.65	0.56-0.72	0.63	0.56-0.69	0.63	0.54-0.70
Negative predictive value	0.6	0.53-0.68	0.61	0.55-0.67	0.57	0.51-0.65
Relative risk	1.53	1.2-1.79	1.48	1.21-1.89	1.43	1.1-1.71
Odds ratio	2.30	1.4-3.34	2.3	1.49-3.54	2.10	1.35-3.02

Table 6. Sensitivity and specificity for cut-off values of arterial stiffness parameters determined by Arteriograph for discriminating coronary artery disease and type 2 diabetes mellitus.
CI: confidence interval.
* cut-off value for PWVao: 10.20 m/s
** cut-off value for AIXao: 33.23 %
*** cut-off value for PWVao: 10.21 m/s

In addition, when ROC analysis was performed in CAD patients not receiving ACEI/ARB vs. control subjects significant improvement in sensitivity and specificity were found for PWVao and AIXao ($p<0.05$) (Table 7).

Variable	**PWVao (m/s) ***		**AIXao (%) ****	
	Value	**95 % CI**	**Value**	**95 % CI**
AUC	0.66	0.56-0.77	0.60	0.51-0.70
Sensitivity	0.69	0.58-0.74	0.61	0.54-0.7
Specificity	0.61	0.54-0.69	0.61	0.54-0.7

Table 7. Sensitivity and specificity for cut-off values of arterial stiffness parameters determined by Arteriograph for CAD patients not taking ACEI/ARB.
CI: confidence interval.
* cut-off value for PWVao: 10.20 m/s
** cut-off value for AIXao: 33.23 %

ROC analysis revealed acceptable sensitivity and specificity results for PWV at a cut off value of 10.20 m/s ($p<0.05$) for the analysis of T2DM vs. healthy control subjects (Fig. 7).

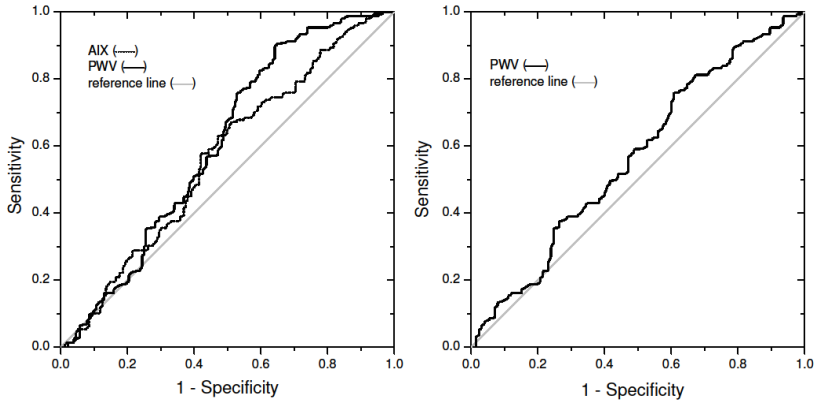

Figure 7.
Receiver-operating characteristic (ROC) curves of the simultaneously recorded aortic augmentation index (AIXao) and pulse wave velocity (PWVao) in case of patients with established coronary artery disease and age-, gender-, mean blood pressure- and heart rate-matched control subjects and ROC curve of the pulse wave velocity (PWVao) in case of patients with T2DM and age-, gender-, mean blood pressure- and heart rate-matched control subjects.

6.3. Discussion

Comparing the CAD and the age-, gender-, blood pressure-, and heart rate-matched control subjects we found that PWVao and AIXao were significantly higher in the CAD group. Therefore, we can suppose that the significantly higher aortic PWV and AIX values are specifically related to the impaired arterial function in the CAD patients. Our findings are supported by the results of Weber et al., who also indicated a very strong relationship between the increased aortic AIX and CAD that was proven by coronary angiography. The relationship between coronary atherosclerosis and aortic PWV was elegantly proven by Kullo and co-workers in a large study assessing the quantity of coronary artery calcium with computed tomography and the aortic PWV with carotid-femoral PWV measurement. The average age of the population studied in their work was very close to ours, thus enhancing comparability with our findings.

Another important observation of our research is that aortic stiffness as measured with PWVao was similarly elevated in the CAD and in the age-, gender-, blood pressure-, and heart rate-matched T2DM group, while T2DM patients showed significantly reduced AIXao when compared to CAD patients. The greatest value of our study is the precise matching of the studied populations that excluded the possible modifying effects of age, gender, blood pressure and heart rate on PWVao and AIXao during the comparison. Taking into consideration that impaired PWVao is the sign of elevated cardiovascular risk, this similarly elevated PWVao could be the evidence that patients with T2DM carry as high risk as patients with known ischemic heart disease. However, the difference in AIXao between the age-, gender-, blood pressure- and heart rate-matched CAD and T2DM patients were striking. The lower value of augmentation index in case of T2DM patients could be explained by the assumption that in several patients with T2DM hyperinsulinaemia could exist, which produces increased sympathetic activity and consequently, lowers the AIX. Furthermore, we

cannot exclude the potential effects of the applied drugs on the AIXao, since several studies showed the beneficial effects of ACEI/ARB, statins, CCB and vasodilatator BB on AIXao and PWVao. According to our results the use of ACEI/ARB was a significant determinant of the stiffness parameters. Our data suggest that pharmacological modulation of the stiffness parameters could also explain the relatively lower AIXao data in the T2DM group.
The ROC analysis in our CAD patient study population advises to use 10.2 m/s as the cut-off value for regional aortic pulse wave velocity. Our finding precisely matches the new recommendation of carotid-femoral PWV (cfPWV) recording, suggesting that the pulse wave analyzer Arteriograph measured PWVao is close to the cfPWV value as it is pointed out by other studies. The sensitivity and specificity results for the Arteriograph are in the acceptable range, however the above mentioned confounding effect of the antihypertensive, antilipid, and oral antidiabetic drugs applied in the CAD, T2DM groups could explain this apparent controversy. Our study proved the pharmacological modulation of the stiffness parameters for ACEI/ARB, resulting in decrease for PWVao and AIX.

7. DISCUSSION

7.1. Invasive validation of the Arteriograph

The methods have been used so far for the determination of arterial stiffness parameters have not become part of the daily clinical routine for several reasons, for instance the time-consuming and complicated nature of the examination. However, the Arteriograph uses a simple cuff as a sensor and the measurement only takes a few minutes.
Compared to previous investigations, we provided more accurate results for assessing SBPao than provided by radial applanation tonometry.
The other important result of the study is revealing the significant correlation between the invasively measured true aortic PWV and the oscillometrically determined PWVao with limits of agreement acceptable for clinical practice.
The surprisingly high agreement between the oscillometrically and invasively measured PWVao may provide data about the reflection site of the aortic pulse wave. According to our findings we can conclude that the forward wave is reflected with a high probability from the area of the aortic bifurcation.

7.2. Comparison of aortic and carotid arterial stiffness parameters in patients with verified coronary artery disease

The principal contribution of the present study is that we found a significant increase of both aortic and carotid PWV and AIx values determined by the Arteriograph and the method of carotid echo-tracking in patients with verified CAD compared to healthy subjects.
Our measurements also reveal a strong correlation of AIx data simultaneously conducted through oscillometric and echo-tracking techniques. With the use of echo-tracking we can gain valuable data about local arterial stiffness that are different from the parameters we can obtain via the intima–media thickness (IMT) measurements.
In this study, we did not find any significant correlation when local and systemic arterial stiffness parameters were compared to the coronary SYNTAX Score, which might mean that parameters of arterial stiffness can not provide any information about the arterial damage in the coronary vessels.

7.3. Comparison of arterial stiffness parameters in patients with coronary artery disease and diabetes mellitus using Arteriograph

Comparing the CAD and the age-, gender-, blood pressure-, and heart rate-matched control subjects we found that PWVao and AIXao were significantly higher in the CAD group, which is in good agreement with earlier studies. Another important observation of our research is that aortic stiffness as measured with PWVao was similarly elevated in the CAD and in the age-, gender-, blood pressure-, and heart rate-matched T2DM group, while T2DM patients showed significantly reduced AIXao as compared to CAD patients. The ROC analysis with an acceptable sensitivity and specificity in our CAD patient study population advises using 10.2 m/s as the cut-off value for regional aortic pulse wave velocity that matches the new recommendation of carotid-femoral PWV (cfPWV) recording.

8. CONCLUSION

We have demonstrated that the parameters (Aix, SBPao and PWVao) measured by Arteriograph, using an oscillometric occlusive method, showed considerably strong agreement and correlation with the values recorded with invasive measurements, and the observed limits of agreement are acceptable for the clinical routine. Our results suggest that the PWVao values measured by the Arteriograph are close to the true aortic PWV determined invasively.

We also found a strong correlation between the stiffness parameters measured with the Arteriograph and those obtained with the echo-tracking method.

We have revealed a significant impairment of arterial stiffness measured as increased PWVao in patients with CAD and T2DM, which reflects premature arterial damage. The cut-off value for PWVao measured by Arteriograph is in good correlation with the recently published recommendation of cfPWV recording. However, the clinical significance of AIXao as a useful vascular stiffness marker in T2DM group was not supported in our study design.

Our findings encourage the implementation of arterial stiffness and function measurements in daily clinical routine in high cardiovascular risk patients with CAD and T2DM as well as in the apparently healthy population suspected for CAD.

9. NOVEL FINDINGS

1. We have validated a new, oscillometric device, the Arteriograph, that measures arterial stiffness noninvasively.
2. We have proven in a large number of patients that the Arteriograph-derived stiffness parameters are in close agreement with those detected during cardiac catheterization.
3. We have demonstrated that the aortic pulse wave velocity measured with the Arteriograph is closed to the true aortic PWV detected invasively.
4. We have found a close relationship between regional and local arterial stiffness parameters measured with the Arteriograph and the echo-tracking method, respectively.

5. We did not find any significant correlation between local and systemic arterial stiffness parameters and the coronary SNTAX Score, consequently stiffness parameters can not provide information about the precise arterial damage in the coronary vessels.
6. We have revealed a significant impairment of arterial stiffness, measured as increased aortic pulse wave velocity in patients with CAD and T2DM.
7. We have established the cut-off value for PWVao measured by Arteriograph that is in good correlation with the recently published recommendation of cfPWV recording.

10. PUBLICATIONS OF THE AUTHOR

Impact factor of original papers: **23.446**

10.1.Original research publications related to the thesis

Zsófia Lenkey, Miklós Illyés, Renáta Böcskei, Róbert Husznai, Zsolt Sárszegi, Zsófia Meiszterics, Ferenc Tamás Molnár, Gábor Hild, Attila Cziráki and Balázs Gaszner. Comparison of arterial stiffness parameters in patients with coronary artery disease and diabetes mellitus using Arteriograph. **Physiol Res.** 2014; 63:429-37. **IF: 1.487**

B. Gaszner, Zs. Lenkey, M. Illyés, Zs. Sárszegi, I.G. Horváth, B. Magyari, F. Molnar, A. Cziráki. Comparison of aortic and carotid arterial stiffness parameters in patients with verified coronary artery disease. **Clin Cardiol**. 2012; 35:26-31. **IF: 1.834**

Horvath IG, Nemeth A, Lenkey Zs, Alessandri N, Tufano F, Kis P, Gaszner B, Cziraki A. Invasive Validation of Arteriograph – a New Oscillometric Device For Measuring Augmentation Index, Central and Brachial Blood Pressure and Aortic Pulse Wave Velocity Simultaneously. **J Hypertens**. 2010; 28:2068-75. **IF: 3.98**

Ratgéber L, Lenkey Zs, Németh Á, Hidvégi E, Husznai R, Verzár Zs, Illyés M, Bódis J, Cziráki A. The effect of physical exercise on arterial stiffness parameters in young sportsmen. **Acta Cardiologica** 2015; 70(1):59-65 **IF: 0.561**

10.2.Letters related to the thesis

Horvath IG, Lenkey Z, Cziraki A. Validation of the Arteriograph working principle: questions still remain. **J Hypertens**. 2011; 29:620. **IF: 4.021**

Horvath, Ivan G.; Lenkey, Zsófia; Cziráki, Attila: Reply to the letter of B. Trachet et al. **J Hypertens** 2011; 29:1663-1664. **IF : 4.021**

10.3.Original publications not related to the thesis

Aradi D, Vorobcsuk A, Lenkey Zs, Horváth IG, Komócsi A. Low platelet disaggregation predicts poor response to 150 mg clopidogrel in patients with elevated platelet reactivity. **Platelets**. 2010; 21:1-10. **IF: 2.117**

Ajtay Zénó, Cziráki Attila, Németh Ádám, Lenkey Zsófia, Horváth Iván, Szabados Sándor. Az aszimmetrikus dimetil-arginin (ADMA) változása koronáriabetegekben revaszkularizáció során. **Gyermekgyógyászat** 2010; 61. évfolyam 6. szám

Attila Cziráki, Zénó Ajtay, Ádám Németh, Zsófia Lenkey, Endre Sulyok, Sándor Szabados, Nasri Alotti, Jens Martens-Lobenhoffer, Csaba Szabó, Stefanie M. Bode-Böger. Effects of coronary revascularization with or without cardiopulmonary bypass on plasma levels of asymmetric dimethylarginine**. Coronary Artery Disease** 2011; 22:245-52. **IF: 1.237**

Erzsebet Hidvegi, Miklos Illyes, Bela Benczur, Renata M Bocskei, Ratgeber Laszlo, Zsofia Lenkey, Ferenc T Molnar, Attila Cziraki. Reference Values of Aortic Pulse Wave Velocity in a Large Healthy Population aged between 3 and 18 Years. **J Hypertens** 2012; 30:2314-21. **IF: 3.806**

Komócsi A, Vorobcsuk A, Faludi R, Pintér T, Lenkey Z, Költo G, Czirják L. The impact of cardio-pulmonary manifestations on the mortality of systemic sclerosis: a systematic review and meta-analysis of observational studies. **Rheumatology** 2012; 51:1027-1036. **IF: 4.212**

Ádám Németh, László Hejjel, Zénó Ajtay, Lóránd Kellényi, Andor Solymos, Imre Bártfai, Norbert Kovács, Zsófia Lenkey, Attila Cziráki, Sándor Szabados. The assessment of neural injury following open heart surgery by physiological tremor analysis. **Archives of Medical Sci** 2013; 9:40-6. **IF: 1.89**

Hidvégi EV, Illyés M, Molnár TF, Böcskei MR, Benczúr B, Lenkey Zs, Cziráki A. Az aorta pulzushullámterjedési sebesség referencia értékei nagy egészséges 3-18 éves korú populációban. **Gyermekgyógyászat** 2014; 64:287-291.

Varga-Szemes A, Simor T, Lenkey Z, van der Geest RJ, Kirschner R, Toth L, Brott BC, Elgavish A, Elgavish GA. Infarct density distribution by MRI in the porcine model of acute and chronic myocardial infarction as a potential method transferable to the clinic. **International Journal of Cardiovascular Imaging** 2014; 30**:**937-948. **IF: 2.322**

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