Measurements of Flow Mediated Dilation and Shear Rate in the Radial Artery Using 20 MHz Ultrasonic System in Patients with Coronary Artery Disease

NARODOWY INSTYTUT GERIATRII, REUMATOLOGII I REHABILITACJI im. prof. dr hab. med. Eleonory Reicher

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INTRODUCTION

The process of development of atherosclerosis is preceded by endothelial dysfunction of blood vessels and the development of a local inflammatory process. The endothelium is involved in the regulation of vessel tone, blood cell adhesion to vessel walls, the formation of blood thrombi, and modification of anticoagulant and anti-inflammatory properties.
Early and accurate assessment of endothelial function may help in understanding the etiology of these diseases and in determining the effectiveness of treatment of vascular diseases. Arriving at such an assessment begins with an understanding of the endothelial response to blood flow. As blood flows through the artery, shear stress is generated on the surface of endothelial cells. In response to the blood flow-induced shear stress, which is registered as a mechanical stimulus, numerous vasoactive mediators are released from the endothelial cells. It is established that flow-mediated dilation (FMD) of the vessel correlates well with the release of NO. The percentage change in FMD is determined by comparing the diameter of the vasodilated vessel after reactive hyperaemia with the baseline diameter before vessel occlusion.
It was demonstrated that a change of the magnitude of FMD not only reflects the endothelial function but also depends on the value of the applied shear stress stimulus resulting from reactive hyperaemia and different flow velocity gradients in the vicinity of the endothelial lining. The total value of the stimulus corresponds to the area under the shear stress curve or approximately to the shear rate curve.
The precision of artery diameter measurements depends directly on the axial resolution of the applied ultrasound sound (US) scanner. The axial resolution of the standard US scanners working at 7.5–12 MHz, is limited to about 0.3–0.4 mm, which is close to the expected dilation of the brachial or radial arteries and thus severely biasing the results. This is why we have introduced a 20 MHz linear array, with its superior axial resolution close to 0.1 mm, to the FMD measurements.

AIM

The aim of this study was assessment of FMD for radial artery (FMDr) using 20 MHz ultrasonic system in patients with coronary artery disease.

MATERIAL AND METHODS

Participants.

Our pilot studies involved two groups: Group I consisted of 27 healthy volunteers, 15 men and 12 women (20–71 yr old), and Group II consisted of 17 patients, 14 men and 3 women (36–77 yr old), with chronic coronary artery disease (CAD). The diagnosis of chronic CAD was based on the presence of symptoms of stable angina or a positive myocardial ischemia stress test (confirmed >50% epicardial coronary stenosis in one or more epicardial coronary arteries). Patients presenting with unstable angina and myocardial infarct were excluded.

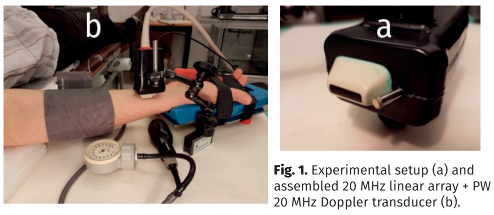

Fig. 1. Experimental setup (a) and assembled 20 MHz linear array + PW 20 MHz Doppler transducer (b).

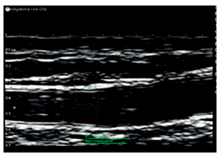

Fig. 2. Tracking of the temporal radial artery (at the intima/blood interface) dilation using Brachial Analyzer software.

An experimental setup consisted of a 20 MHz SonixTouch-Research ultrasound scanner (Analogic Corporation, Peabody, MA, USA) with a 20 MHz linear probe, combined with a single-element 20-MHz multigate Doppler (developed in our laboratory) probe, Fig. 1a. The Doppler probe was fixed in a plastic housing, together with the linear array, providing the flow measurement within the imaging plane. The angle between the Doppler beam and the scanning beam was 68° (Fig. 1b). Up to 20 gates were positioned in a typical radial artery. The Doppler shift in each of the gates was calculated and displayed on the monitor. For the further processing, only the FFT Doppler spectrum from the central blood stream, corresponding to the maximum flow velocity V_{max}, was selected (Fig. 2).

Protocol.

Subjects were instructed to fast starting from the night before testing and to refrain from ingesting alcohol or caffeine or taking any vasoactive medications on the day of testing and the day preceding it. Each patient was placed in a supine position for 10 min of rest in a quiet setting before the measurement.

In the next step, the forearm cuff was inflated to a pressure 50 mm higher than the systolic pressure at rest. The blood flow arrest lasted for 5 min and then the cuff was released. The recordings of radial artery diameter and blood flow velocity were resumed 10 seconds before cuff deflation and continued for 3 minutes.
The sequences of ultrasound images were recorded at 25 frames/s in RAW format and were next converted to AVI format. The AVI files were then analysed offline, using brachial analyser (BA) software (Medical Imaging Applications, LLC, Coralville, IA).
The BA software makes it possible to track the time variations of the vessel diameter in a chosen region of the ultrasound scan of the artery (Fig. 3). The measurements of each vessel diameter were repeated twice by two experienced ultrasonographers, and the variability between results did not exceed 2.8%. For further calculations the mean values of the two measurements were used. The post-deflation SR was calculated as four times the maximum velocity recorded along the central flow stream in the artery divided by the baseline vessel diameter and multiplied by the time from cuff deflation to the time when the peak diameter was reached.
The flow rate recorded during this time was then used to calculate the accumulated shear rate. Calculations of FMDr and time to peak artery dilation were based on standardized algorithms applied to data that had undergone automated edge-detection and wall tracking. Blood flow velocities were recorded for 2 min after releasing the cuff pressure, and the SR values were calculated offline (Fig. 3-6).

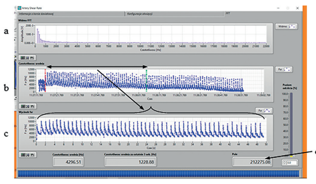

Fig. 3. (a) Typical recording of the Doppler instantaneous spectrum, (b) 120 s of mean Doppler frequency and (c) expanded view of mean Doppler frequency from cuff release to time of maximum vessel dilation (time TS). (d) The value of AUC under the Doppler frequency (or velocity) recording is automatically calculated in [mm].

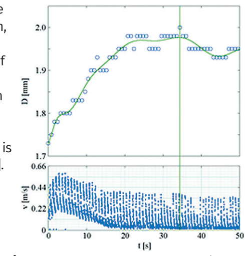

Fig. 6. An example of the calculation of the artery diameter D dilation and 50 sec of Doppler flow/shear rate recording. SR_{AUC} calculated in the time span (34 sec) between releasing the cuff and the time of artery peak dilation.

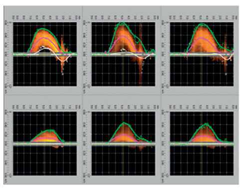

Fig. 4. Typical recordings of the spectral profiles in radial artery.

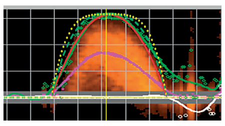

Fig. 5. Velocity profiles, the red solid lines is a parabolic profile, n=2.

RESULTS

The following data were analysed to find the level of correlation between them: age, BMI, baseline diameter D_b, SR, FMDr, and FMDr/SR. All the correlation coefficients were calculated with the confidence level of 0.95, providing the comparison between the values of the linear relationships for all pairs of data sets. The highest Pearson coefficient was found for the FMDr–age pair and was equal to $r = -0.76$. Nearly the same level of correlation was found for the pair of the FMDr and FMDr/SR data sets ($r = 0.71$). The correlations exist between FMDr and BMI and between FMDr and D_b, are equal to $r = 0.7$ and $r = -0.58$, respectively. The lowest correlation was found for SR combined with other data sets; for example, $r = -0.48$ between SR and FMDr/SR.
The baseline diameter D_b is not significantly correlated with any of the other measured parameters. Because of this, it can be used as an independent variable in further study of multivariate associations between data sets.
Taking into account the high correlation between FMDr, FMDr/SR, and age, two relationships, FMDr–age and FMDr/SR–age, were considered so as to determine whether they might be helpful in differentiation of the two examined groups.
It is worthy of note that the younger volunteers from group I (below 35 yr old) exhibited rather high variability in FMDr values compared to the older volunteers; however FMDr values were not lower than 8% for any of the subjects from group I. The cut-off value of FMDr = 7.5% illustrates that only 3 out of 17 patients from group II had FMDr values exceeding this value; one had FMDr equal to 8% and two had FMDr equal to 11 and 15%, respectively.

variable	mean/ median	all (n=44)	group I (n=27)	group II (n=17)	tests
D_b [mm]	mean±sd	2.50±0.42	2.36±0.40	2.75±0.32	Welsh test,
	range	1.73-3.37	1.73-3.37	2.30-3.27	p-value < 0.0001
D_{peak} [mm]	mean±sd	2.73± 0.39	2.64±0.40	2.85± 0.30	Welsh test, p-value >0.05
	range	1.97- 3.57	1.97- 3.57	2.37-3.26	
FMDr [%]	mean±sd	11.11±6.98	15.26±4.90	4.53±4.11	MWW test, p-value <0.001
	range	0-25	8-25	0-15	
SR (10^4)	mean±sd	4.12±2.85	4.27±2.36	3.88±3.56	MWW test, p-value > 0.05
	range	0-14.50	1.22-9.30	1.50-14.50	
FMDr/SR (10^{-4})	mean±sd	3.9±4.20	5.4±4.6	1.4±1.2	MWW test, p-value <0.001
	range	0.0-18	0.9-18	0-5	

MWW - Mann-Whitney-Wilcoxon test

Table 1.
Summarized results for selected variables in the studied groups of subjects

The Pearson correlation coefficient between SR and FMDr/SR was $r = -0.48$, which is less than 0.5. The nonlinear character of FMDr/SR versus SR relation was confirmed.
The range, mean±SD, median, CI's of measured and calculated variables for three groups (group of all subjects, group I and group II) as well as p-value of tests performed for groups' differentiation, are summarized in Table 1.
The variable D_b for both groups can be treated as normally distributed; the Welch two-sample t-test was used, and it confirmed that the difference between groups is statistically highly significant, with a p-value < 0.001. We did not use this parameter alone in our discussion of differentiation between two groups, but rather we used the relative measure of dilation, FMDr, because of its confirmed diagnostic significance.
The time span, TS, from cuff release until peak dilation, is not a useful parameter from the point of view of differentiation of groups. The Welch test confirmed that TS was statistically indistinguishable between the two groups; the hypothesis that the difference in means is equal to 0 could not be rejected, with a p-value > 0.05.
The FMDr values for group II could not be considered normally distributed, but the equality of scales was confirmed by the Ansari-Bradley test with a p-value > 0.05. So, the differentiation between the groups was examined by the MWW and KS tests, and both p-values were much less than 0.001. This allows us to consider the true differences between distributions to be highly statistically significant.

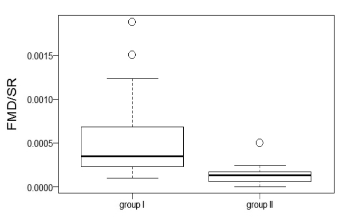

Fig. 7. FMDr/SR values for Group I healthy volunteers and Group II patients with CAD

The shear rate, SR, does not differentiate the healthy volunteers from CAD patients: the two groups do not have significantly different distributions, and both the MWW and the KS test have p-values much larger than 0.05 (see Table 1). FMDr/SR values were also used for differentiation between the two groups. Although the data from both groups are not normally distributed, as previously, the p-value > 0.05 of the Ansari-Bradley test suggests that the MWW test can be used to measure differences in medians. Indeed, the FMDr/SR values for both groups are highly significantly different, with p-valuesof the MWW and KS tests much smaller than 0.001 (Fig. 7).

CONCLUSION

In the pilot study of the FMDr of the radial artery, we have demonstrated that using high frequency 20 MHz scanning and Doppler ultrasound allowed us to precisely record both the blood flow and the dilation of the radial artery. An ultrasound scanner combined with a 20 MHz multigate Doppler probe was used in this pilot study. The FMDr results revealed that the Radial Artery Reactive Response in healthy volunteers changed from 8 to 25% with a mean of 15%, while in the group of CAD patients, the mean changes did not exceed 5%.

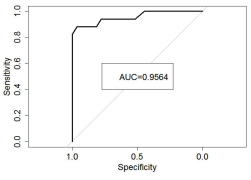

Fig. 8. ROC–AUC for SR normalized FMDr

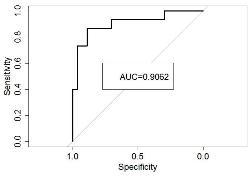

Fig. 9. ROC–AUC for FMDr

In a group I a mean maximal increase in radial artery diameter was 15±4.8% and SR normalized FMDr was 5.36±4.83·10^{-4}. In a FMDr was 4.6± 4% and SR normalized FMDr was 1.3± 0.89·10^{-4}. Both parameters allowed to separate both group with a ROC–AUC equal 0.96 and 0.90, respectively (Fig. 8–9).
Additionally, our results do not justify the need to use the FMDr/SR ratio as an effective parameter for prediction of atherosclerotic lesions.

Conflicts of interest: none