Flow mediated endothelium function: advantages of an automatic measuring technique

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Abstract. The objective of this work is to show the advantages of a non invasive automated method for measuring flow mediated dilation (FMD) in the forearm. This dilation takes place in answer to a shear tension generated by the increase of blood flow, sensed by the endothelium, after the liberation of an occlusion sustained in the time. The method consists of three stages: the continuous acquisition of images of the brachial artery using ultrasound techniques, the pulse to pulse measurement of the vessel’s diameter by means of a border detection algorithm, and the later analysis of the results. By means of this technique one cannot only obtain the maximum dilation percentage (FMD%), but a continuous diameter curve that allows to evaluate other relevant aspects such as dilation speed, dilation sustain in time and general maneuver performance. The simplicity of this method, robustness of the technique and accessibility of the required elements makes it a viable alternative of great clinical value for diagnosis in the early detection of numerous cardiovascular pathologies.

1. Introduction
Flow mediated dilation (FMD) describes the increase in diameter of a blood vessel, generally the brachial artery, in answer to a shear increase in the shear associated to blood flow. The mechanical stimulus on the endothelium generates liberation of nitric oxide (NO) responsible for the dilation [1]. The answer of the arterial diameter to the change of flow can be measured in a non invasive way by means of ultrasound, after an ischemia caused by a temporary forearm occlusion. The dilation magnitude, expressed as the percentage change of the dilation diameter after 60 seconds of having liberated the occlusion and the basal diameter (FMD%), is traditionally used to evaluate endothelium response [2]. The conventional protocol assumes that it is around this time that maximum dilation takes place. Diameter measurements are carried out manually from a still image using the available cursors in the ultrasound equipment. These techniques revealed to have important methodological deficiencies and their use is discouraged [2].

In subjects without previously detected arterial illnesses, the magnitude of the FMD% turned out to be inversely related to diverse cardiovascular risk factors and subclinical atherosclerosis [3, 4]. Recently a new monitoring software that allows the continuous measuring of brachial artery diameter during the evolution under hiperemia has been developed [5]. The present work analyzes the advantages of the automatic continuous detection method based on ultrasound images, and its influence in FMD measurement. The most relevant aspects of this new method will be studied.
2.  Techniques

2.1.  Acquisition

Images are obtained from a patient placed in horizontal position with his arm on a stable support that allows to properly positionate an ultrasound probe in order to visualize the brachial artery. The acquisition requires an echographer in B mode and a linear transducer for vascular application (7 MHZ). Digitalization is carried out continuously from a PAL image in 8 bits format using a PC (Pentium IV, 1GHz, 256MB RAM). For the sequences acquisition, a capture and storage Software specifically developed for this end is used. This program allows capturing real time images, converting them to AVI video format and processing them automatically. The captured image has a size of 768x576 pixels and is acquired at a frequency of 12.5Hz. A properly positioned internal rectangle determines the region of interest (ROI). It should be selected in order to obtain the distal and proximal walls of the artery in a parallel and horizontal position. Once the sequence is captured, program offers a pre-processing possibility to adjust brightness, contrast, rotations, and to measure the instantaneous diameter and intima media thickness (IMT). Figure 1 shows a preliminary arterial wall detection, where column profile averages of the pixels inside the ROI are presented to the right.

![Figure 1: Ultrasound image of the brachial artery. Inner rectangle represents the ROI. Right: Intensity pixel profile; diameter limits are shown.](image)

2.2.  Processing

Processing software receives a sequence in AVI format and detects, picture by picture, the evolution of the registered arterial diameter, at a subpixel level. It uses a border detection algorithm inside a new user determined ROI. This algorithm equalizes the image histogram, averages the columns, resulting in a single intensity profile average (Fig.1). This profile is derivated and the interfaces that determine the diameter are highlighted. By means of probabilistic analysis, the pixels with highest probability of belonging to the borders of the artery are selected. Details about this algorithm have been previously published [5].

Applying the described detection for each image of a sequence, this module of numeric processing allows calculating the temporary evolution of the diameter and plotting the results. No ECG is needed as diastolic diameter points can be automatically detected and used to follow the temporary evolution of the dilation curve.

2.3.  Shear-rate: Stimulus quantification

Tension on the vessel wall (S) in each instant is obtained multiplying blood viscosity (µ) by the shear rate (SR) corresponding to the speed gradient of the fluid in the artery:

\[ S = \mu \frac{dv}{dr} \]  

(1)
where \( v \) is blood speed for each point \( r \) of the arterial radius. SR on the wall of the artery of \( R \) radius depending of the central speed averages \( v_c \) is calculated as:

\[
\text{Shear Rate} = \frac{4v_c}{R}
\]  

(2)

Central speed in an artery can be obtained by means of ultrasound Doppler. The Doppler effect is the apparent change in the frequency received due to the movement between the sound source (blood flow) and sound receiver (piezoelectric transducer). The Doppler produces an audible sign as well as a graphic representation of the flow (Spectral Wave) that can be analyzed and quantified by commercial ecographers. These values can be recovered manually from the on-screen values and stored for their processing.

2.4. Full maneuver description

Details of the measuring techniques can be found in international recommendations [2]. Briefly, the study begins with the patient in horizontal position, a sphygmomanometric cuff in his right forearm and the vascular probe of the ecographer in a fixed position on the brachial artery. A 10 second basal diameter acquisition is carried out. Once the patient's systolic pressure is registered by means of a gauge placed in the left arm, a 5 minute occlusion is performed. This is carried out inflating the cuff to a suprasistolic pressure, 50 mmHg above the systolic one registered. During the occlusion, sequences of 10 seconds every 1 minute are registered. Thirty seconds before the liberating the occlusion, the acquisition of a 3 minute sequence begins that will register hiperemia as a response to the shear stimulus sensed by the endothelium. The Doppler measurement cursor is also positioned on the ecographer's screen to acquire the registrations of instantaneous speed during hyperemia. Ten minutes of rest are necessary before this procedure, to assure the return to patient's basal conditions. Once registered, the sequence is digitally stored and the results are analyzed. A typical example of the results can be observed in Fig.2. The program automatically informs FMD percentage dilation on screen, together with the evolution curves.

A period of 10 minutes rest is required again before next maneuver. Without changing position, measurement of flow-independent endothelial function is carried out. This is induced using sublingual or spray nitroglycerine (NTG) [2]. This time, without cuff, the 5 minutes after NTG administration are registered. This induced dilation depends on the activation of vascular smooth muscle. Differences in the evolution of dilation curves are outlined in Fig. 3.

![Figure 2. Time evolution of arterial diameter before and after hyperaemia with superposition of diastolic flow speed in the vessel’s centre.](image)

3. Results
Typical results of a complete measurement maneuver of the endothelial function by ultrasounds methods can be summarized in Figs. 1, 2 and 3. In the first one, a window of the acquisition software is observed. A horizontal section of the brachial artery framed by the ROI can be appreciated. The result of the border detection algorithm creates a gray level profile that is shown in the right side of the image. The maxim derivates of the profile (crosses) are the indicators of an abrupt interface change. The difference between the position of distal and proximal walls corresponds to arterial diameter.

![Flow Mediated Dilation (FMD)](image1)

![Endothelium Independent Dilation (NMD)](image2)

**Figure 3.** Up.: Endothelium mediated dilation (NO). Down: Sublingual NTG mediated dilation.

Two sequences are exported to the processing module, corresponding to the basal measurement (10 seconds) and the hyperemic one (3 minutes) respectively. Figure 2 shows both sequences in function of time. The graph allows distinguishing clearly three stages in the hyperemia region. The first one is a plateau of approximately 30 seconds where diameter doesn't present modifications. This diameter is denominated post-occlusion diameter. During this time, SR is maximum and the endothelium stimulus is taking place together with the liberation of vessel dilators substances. Next, a second stage of marked increment of the arterial diameter is observed until it reaches a maximum (between 30 and 60 seconds after cuff liberation). Finally, diameter gradually recovers to basal values in a slow descendent curve. The overlapping of the stimulus (SR) with the dilation of the diameter allows clearly analyzing the phenomenon's cause and effect.

FMD maneuver should be compared with another that allows the process to become independent of endothelium effect. Using a vessel dilator drug, the third figure shows the differences. In this case, diameter's growth is homogeneous and shows smooth muscle's dilation capacity in response to a pharmacological stimulus. With regard to the dynamic maneuver of FMD, the effect of the NTG is more gradual and remains at the maximum value for a longer period of time. Also, the dilation reached with NTG is usually significantly grater.

**4. Discussion**

The method presented in this work presents multiple advantages with regard to the conventional method, which manually calculates FMD by using two punctual dilation values. Actually, manual methods are not recommended due to their high inter-intra observer dependence [2-5]. Automatic method of acquisition allows a beat to beat register, having the clear advantage of obtaining a continuous curve that shows diameter time dependant evolution as an answer to stimulus. This allows the user to unequivocally obtain the value of maximum dilation taking place after occlusion is liberated. The importance of this is fundamental in patients that reach maximum dilation before or
after the established time (around 60 seconds), resulting in an erroneous FMD% value. For example, in children, it has been demonstrated that the time for maximum dilation may vary significantly [6].

The traditional technique also requires achieving a perfect synchronization with the ECG signal in order to determine diastolic diameter. The method presented in this work solves this limitation by determining pulsating diameter in a single beat, allowing easily differentiating diastolic and systolic phases without an ECG. This makes the whole maneuver easier for the user and eliminates errors that can be committed by synchronization problems, at the same time that it allows a detailed distensibility analysis beat by beat.

The conventional way of measuring diameter recommends the observer to take an average of three manual measurements. Although averaging tries to reduce error, the elections of diameters depend on each observer. The same observer studying the same still image could obtain different results in two different occasions. The automation of the acquisition and processing module allows not only the exact determination of maximum dilation time, but makes FMD measurement user-independent [5]. This way, an important error source is reduced because the observer no longer determines diameter manually, neither carries out the calculation of FMD. Evidently, the variability in FMD is biologically unavoidable. FMD results fluctuate from patient to patient, and even an intrinsic and physiologic variability for the same patient exists. A patient that undergoes two consecutive studies will naturally result in different dilation values. This is due to the diverse factors that influence endothelium function, from which diet, menstruation in the woman, circadian rhythms, glucemia, stress level, among others, can be mentioned. There are also methodological circumstances that tend to limit intrinsic dispersion, among which we can mention automation of the detection method, correct ecographic probe positioning on the subject, room temperature and technical operators related aspects. This way, the presented method allows diminishing some of these methodological difficulties to assure an increased result repeatability.

On the other hand, being able to obtain a continuous diameter evolution curve allows investigating other relevant aspects such as diameter growth speed (curve slope), dilation sustained in time, return speed to basal condition and general maneuver performance. Morphology of the curve can also help to identify patients with previously undetected pathologies.

FMD% value alone does not always constitute a relevant indicator, since a low result (FMD <5%) can respond to either an endothelial dysfunction or an insufficient stimulus. In this aspect the presented method is superior since a continuous dilation register makes feasible the temporary comparison with that of the shear caused by hyperemia, allowing corroborating if quantitative data is consistent with qualitative results. At the present time, two aspects that have strong influence over dilation percentages are under serious discussion. On one hand it was demonstrated that absolute basal diameters influence the FMD% [7]. Smaller diameters cause higher dilations. This can be due partly to an increased SR stimulus. However, the topic is still under discussion, but should be kept in mind especially when groups of patients are used where the predominance of women can bias results because of the fact that they have smaller arteries. The second discussion topic is the normalization of FMD% dilation effects with regard to stimulus. Evidently, for a patient that achieved a higher dilation, it is indispensable to know if that was caused by a better endothelial system answer or simply to a higher stimulus caused during hyperemia. At the present time its strongly recommended to normalize dilation percentage with SR curves peak. Recent works even show that the area under the stimulus curve is a better indicator of its characteristics than its peak value [8].

5. Conclusions

This paper discusses the advantages of having a method of automatic detection of arterial diameter from ultrasound images, to be applied to the measurement of flow mediated endothelial function. The main advantage is the capacity to obtain a continuous evolution curve of the diameter of the brachial artery during hyperemia. This curve allows objectively measuring responses to stimulus, identified as the shear on the wall. Automatic methods allow the reduction of methodological errors, to obtain user-independent results and to assure an appropriate repeatability to apply this technique for epidemic
studies. Finally, the importance of factors like the studied artery size influence on dilations, as well as the need to normalize the observed effects in response to the corresponding stimulus, are outlined.

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References
[1] R. Joannides, W.E. Haefeli, y L. Linder, “Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo”, Circulation, 91:1314–9, 1995.
[2] M.C. Corretti, T.J.Anderson, E.J. Benjamin, D. Celermajer, F. Charbonneau y M.A Creager, “Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force”, J Am Coll Cardiol, 16;39(2):257-65, 2002.
[3] S. Laurent, P. Lacolley, P. Brunel, B. Laloux, B. Pannier y M. Safar, “Flow-dependent vasodilation of brachial artery in essential hypertension”, Am J Physiol, 258:H1004–11, 1990.
[4] D.S. Celermajer, K.E. Sorensen y V.M. Gooch, “Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis”, Lancet, 340:1111–5, 1992.
[5] D. Craiem, G. Chironi, J. Gariepy, J. Miranda-Lacet, J. Levenson y A. Simon. “New monitoring software for larger clinical application of brachial artery flow-mediated vasodilatation measurements”, J Hypertens, 25(1):133-40, 2007.
[6] M.J. Jarvaisalo, T. Ronnemaa, I. Volanen, T. Kaitosaari, K. Kallio, J.J. Hartiala, K, Irjala, J.S. Viikari, O. Simell y O.T. Raitakari, “Brachial artery dilatation responses in healthy children and adolescents”, Am J Physiol Heart Circ Physiol, 282(1):H87–92, 2002.
[7] H.A. Silber, P. Ouyang, D.A. Bluemke, S.N. Gupta, T. K. Foo y J.A.C. Lima, “Why is flow-mediated dilation dependent on arterial size? Assessment of the shear stimulus using phase-contrast magnetic resonance imaging”, Am J Physiol Heart Circ Physiol, 288:822-828, 2005.
[8] K.E. Pyke y M.E. Tschakovsky. “Peak vs. total reactive hyperemia: which determines the magnitude of flow-mediated dilation?”, J Appl Physiol,102(4):1510-9,2007.