Comparison of two automatic methods for the assessment of brachial artery flow-mediated dilation
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Objectives Brachial artery flow-mediated dilation (FMD) is associated with risk factors providing information on cardiovascular prognosis. Despite the large effort to standardize the methodology, the FMD examination is still characterized by problems of reproducibility and reliability that can be partially overcome with the use of automatic systems. We developed real-time software for the assessment of brachial FMD (FMD Studio, Institute of Clinical Physiology, Pisa, Italy) from ultrasound images. The aim of this study is to compare our system with another automatic method (Brachial Analyzer, MIA LLC, IA, USA) which is currently considered as a reference method in FMD assessment.

Methods The agreement between systems was assessed as follows. Protocol 1: Mean baseline (Basal), maximal (Max) brachial artery diameter after forearm ischemia and FMD, calculated as maximal percentage diameter increase, have been evaluated in 60 recorded FMD sequences. Protocol 2: Values of diameter and FMD have been evaluated in 618 frames extracted from 12 sequences.

Results All biases are negligible and standard deviations of the differences are satisfactory (protocol 1: $-0.27 \pm 0.59\%$; protocol 2: $-0.26 \pm 0.61\%$) for FMD measurements. Analysis times were reduced ($-33\%$) when FMD Studio is used. Rejected examinations due to the poor quality were 2% with the FMD Studio and 5% with the Brachial Analyzer.

Conclusions In conclusion, the compared systems show an optimal grade of agreement and they can be used interchangeably. Thus, the use of a system characterized by real-time functionalities could represent a referral method for assessing endothelial function in clinical trials. J Hypertens 28:000–000 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Introduction Flow-mediated dilation (FMD) of the brachial artery is the most widely used technique for assessing endothelial function in humans. This noninvasive method, introduced in 1992 [1], involves measurement of diameter changes induced by endothelial release of nitric oxide mediated by increased shear stress [2]. Shear stress increase is produced by reactive hyperemia which occurs when a sphygmomanometer cuff placed on the forearm, distal to the brachial artery, is inflated to 200–300 mmHg and subsequently released after 5 min [3].

Reduced FMD has been documented in the presence of cardiovascular risk factors and disease [4] and it is predictive of cardiovascular events, independently of traditional cardiovascular risk factors [5,6].

Some practical challenges still limit its use in routine clinical practice [3,6,7], including the need of highly trained operators, the special care required to minimize the effect of environmental and physiological influences [8] and the overall adherence to a rigorous protocol for improving accuracy and reproducibility. In particular, automated systems, based on image processing algorithms to measure brachial artery diameter, are fundamental to obtain the best FMD reproducibility [9].

At the present time, most of the automatic systems for FMD assessment are, however, still based on postprocessing analysis thus only working offline. On the contrary, real-time analysis offers several advantages enhancing reliability and precision of FMD measurement. Mainly, a real-time feedback signal, which is generated during the scan acquisition and strictly related to the algorithm performance, could continuously inform the operator about the quality of the ultrasound images. Consequently, the number of examination rejected due to low-quality postprocessing analysis could be reduced. Moreover, another advantage of real-time analysis is the reduction in time spent in analyzing the images after the acquisition.
The aim of this study was to evaluate the agreement between two different automatic systems: the Brachial Analyzer (Medical Imaging Applications, Coralville, IOWA, USA), already adopted worldwide by a large number of clinical studies [10,12] and the FMD Studio (Institute of Clinic Physiology, National Research Council, Pisa, Italy) [13,14]. Brachial Analyzer is based on a specific software for Personal Computers that allows an automatic off-line analysis of ultrasound images acquired with a frame grabber, whereas the FMD Studio system is composed of a special-purpose hardware/software device [15], which can be directly connected with any ultrasound equipment enabling the FMD assessment in real time. In order to evaluate the agreement between the two systems, Video Cassette Recording (VCR)-recorded ultrasound sequences of several FMD examinations obtained at the Vascular Physiology Unit were analyzed. Moreover, feasibility and timing requirement for analysis with both systems and was compared.

Methods
Study population
Flow-mediated dilation was assessed in 60 participants, 47 healthy volunteers and 13 type 2 diabetic patients, according to the current guidelines [3]. FMD examinations were both from voluntary healthy people recruited from hospital and academic staff and from diabetic people recruited at the Vascular Physiology Unit. Mean age was 38 ± 13 years, 54% were male and none of the participants were smokers or obese [body mass index (BMI) 25.7 ± 3.3 kg/m²].

All ultrasound image sequences were recorded using VCR.

Experimental procedures
Vascular studies were carried out in a temperature-controlled laboratory (23 ± 1°C). The brachial artery of the nondominant arm was scanned in longitudinal section using an Acuson XP10 ultrasound system and a 10-MHz linear-array transducer supported by a stereotactic clamp. The blood flow of the artery was reduced by means of a 9-cm wide pneumatic cuff placed around the forearm, immediately below the antecubital fossa. After 1 min of baseline flow, the cuff was inflated to 500 mmHg for 5 min and then released, resulting in a brief period of reactive hyperemia. The brachial artery diameter changes in response to increased blood flow were assessed for a further 3 min after cuff deflation. The images were recorded on VCR throughout the whole examination.

With the Brachial Analyzer software the operator draws a region of interest (ROI) in which the best definition of the edges of the vessel are not correctly detected. The analysis is based on an automatic detection of the edge of the vessel performed with a globally optimal graph search border-detection approach [11].

Similarly, using FMD Studio, the approximate position of the edges of the vessel is manually located before starting the examination. After this procedure, an automatic mathematical contour tracking operator [16] locates and tracks the edges, supplying information about quality and time course of measurements in real time [13]. On completion of the analysis, both methods automatically generate a report, with all the recorded measurements.

Two protocols were followed in order to estimate the agreement between the two systems. All scans were performed by an experienced operator, who was blinded to the protocol. Data regarding learning curve and reproducibility of the ultrasound lab have been reported elsewhere [12].

With the first protocol, the global agreement when performing a full FMD examination was assessed. In the second protocol, the differences between the performances of the edge operators [16] on single images were compared.

Protocol 1
B-mode images from the 60 participants were recorded on VCR and simultaneously acquired by means of a frame grabber for analysis of the full image sequences using the Brachial Analyzer software. Subsequently, all the sequences were played on VCR and analyzed with FMD Studio. Mean basal diameter over the first minute, maximum post occlusion diameter and value of the maximum percentage increase in diameter (%FMD) were evaluated by both systems.

Protocol 2
Within a subgroup of 12 cases, individual digital images from basal and postocclusion state (therefore excluding those relative to the cuff occlusion) were analyzed with both systems (618 frames), one by one. The 12 cases have been extracted by means of a computer generated random sequence. General characteristics (age, sex, BMI) of the selected participants reflected those of the whole population. Tracking of the edges and evaluation of the diameter of the vessel on every frame were performed with both systems. Comparison between the measurements of the diameter and the values of diameter dilation, as evaluated by the two systems, was carried out. Diameter dilation values were estimated only for frames belonging to the expansion phase. In this case, the mean value of the diameter evaluated on basal images was considered as the baseline diameter.

Apart from the assessment of the agreement of the two systems, the potential advantages of the real-time
The capabilities of FMD Studio were evaluated. Criteria that has been chosen for this comparison were the mean examination time and the percentage number of rejected examinations. Mean examination time included system calibration, ROI tracing, acquisition and processing of diameter changes during basal condition, ischemia, reactive hyperemia and report generation. Examinations were rejected when the quality of the images were not enough to enable the automatic analysis.

**Statistical analysis**

Data are shown as mean ± standard deviation. The agreement between the two methods was evaluated with Bland-Altman analysis, correlation coefficients and regression analysis. Duration of the study was compared by mean value (less time need would be desirable). A P value less than 0.05 was considered statistically significant.

Statistical software IBM SPSS Statistic 18.0 (© 2009 IBM Corporation) was used for data analysis.

**Results**

**Protocol 1**

In the whole study population brachial artery diameter was 3.16 ± 0.71 mm at baseline and 3.43 ± 0.70 mm at maximal dilation corresponding to a %FMD of 9.1 ± 5.2% in the measurement obtained with the Brachial Analyzer. Diameter was 3.15 ± 0.72 mm at baseline and 3.41 ± 0.70 mm at maximal dilation corresponding to a %FMD of 8.8 ± 5.0% in the measurement obtained with the FMD Studio. Correlation coefficients were 0.997 (P < 0.0001) for the mean basal diameter, 0.996 (P < 0.0001) for postocclusion maximum diameter and 0.988 (P < 0.0001) for %FMD (Fig. 1).

Bland–Altman plots (Fig. 2) show the differences between the two methods for the aforementioned measurements. Bias and standard deviation of the differences were −0.01 ± 0.04 mm for the mean basal diameter, −0.02 ± 0.04 mm for postocclusion maximum diameter and −0.27 ± 0.59% for %FMD.

With Brachial Analyzer %FMD was 9.7 ± 5.5% in healthy individuals and 6.7 ± 3.1% in diabetic patients, whereas with FMD Studio it was 9.4 ± 5.3 and 6.6 ± 2.8%, respectively. Correlation coefficients (healthy 0.995; diabetic patients 0.982), bias and standard deviations (−0.31 ± 0.56%; −0.11 ± 0.65%) for %FMD were comparable in
the two subgroups and similar to those of the entire study population.

Protocol 2
Brachial artery diameter was 3.22 ± 0.51 mm at baseline and %FMD 2.6 ± 4.2% in the measurement obtained with the Brachial Analyzer. Diameter was 3.13 ± 0.51 mm at baseline and %FMD was 2.3 ± 4.1% in the measurement obtained with the FMD Studio. Correlation coefficients were 0.998 (P < 0.0001) for the mean basal diameter and 0.990 (<0.0001) for %FMD (Fig. 3).

Bland–Altman plots (Fig. 4) show the differences between the two methods for the aforementioned measurements. Differences were −0.09 ± 0.03 mm for the mean basal diameter and −0.26 ± 0.61% for %FMD.

The mean analysis time was 21 ± 1 min using Brachial Analyzer and 14 ± 1 min using FMD Studio. The percentages of examinations rejected due to the poor quality were 5% with the Brachial Analyzer and 2% with the FMD Studio.
Discussion

The assessment of the endothelial function with ultrasound has become widely performed and is considered a surrogate for the evaluation of cardiovascular health [6]. Brachial artery FMD determined by ultrasound method is a non-invasive technique with a high grade of tolerability by patients. Moreover, reduced FMD has been associated with cardiovascular risk [17,18], damage [19,20] and events [5].

Major detractors, however, sustain the low reproducibility of the technique and its high operator dependency. To face the above-mentioned problems, various automatic systems have been developed. Brachial Analyzer is one of the most reliable and widespread adopted among these and it has been demonstrated to be less variable in direct comparison with other systems [12]. However, Brachial Analyzer lacks in real-time capabilities which should be included in automatic system for FMD evaluation in order to obtain better precision and reliability, lesser time consumption and a possible reduction of sonographer’s learning curve.

In the present study, we demonstrated that the degree of agreement between the Brachial Analyzer and a new automatic system improved with major features (FMD Studio). An accurate evaluation of the degree of agreement between different measurement systems is of great importance because, when it has been proved that the instrumentation is interchangeable, clinical results from different epidemiological studies can be usefully compared.

In the present study, we demonstrated that the degree of agreement between the two systems is excellent both when single patients are examined (protocol 1) and when the efficiency of the two techniques, in terms of vessel edge location and subsequent diameter evaluation in the same digital images (protocol 2), is considered.

As far as the comparison of the percentage diameter dilation values is concerned, a value of the standard deviation of the differences less than 1% for this parameter leads to a more than satisfactory degree of agreement. Apart from this, both protocols showed a standard deviation of the differences of diameter dilation that is largely below the recommended limit value, suggesting a satisfying degree of agreement, also when assessed in subgroups of healthy individuals or patients with a cardiovascular risk factor such as type 2 diabetes.

Distinguishing features introduced by the new system should be taken into consideration. Interestingly, we verified that the number of examinations rejected due to the poor quality of the images was reduced using FMD Studio as compared to Brachial analyzer, suggesting a real time approach might be more feasible as compared to those with offline features. The FMD Studio works at 25 frames/s ensuring greater reliability against noise since diameter measurements are based on the mean of thousands of frames rather than in ECG gating mode in which only about 1 measure per second is available. The full frame rate modality is a key feature to allow a quicker reaction of the physician when recovering a lost ultrasound image also in absence of real time analysis. Moreover, the FMD assessment is also possible when the ECG signal is noisy or not good enough to mark end-diastolic frames.

Additional advantage of the FMD studio is the real-time capability that provides the sonographer with continuous and immediate visual feedback on the image quality throughout the scan. Hence, the sonographer can interact with the mechanical part of the system and adjust the probe thus maintaining the image quality as optimal as possible.

Therefore, the availability of real-time feedback signals helps physicians could improve the precision and reliability of the acquired measures.

The real-time characteristic is also an advantage when considering the time involved in the study processing. In the present study, FMD Studio saved 33% of time analysis as compared with a very efficient system as the Brachial Analyzer. Moreover, when used routinely, FMD Studio generates instantaneously a complete report with the full time course of the measurements, at the end of the 10-min examination, without the need of additional time for the analysis as for Brachial Analyzer.

Another important characteristic might be represented by the evidence that FMD results obtained with the FMD Studio are comparable working with or without ECG synchronized ultrasound images [13]. This feature is remarkable because it allows the use of less specialized and expensive ultrasound equipments (modules for the generation of ECG gated images are not needed). From the sonographer’s point of view, working with full frame rate images is easier than working with gated images when the operator is requested to maintain a fixed scan plane due to the faster reaction of the sonographer in adjusting the position of the probe.

Finally, the real-time feedback signal during the FMD examination would greatly reduce the sonographer learning curve by allowing a self-evaluation of the quality of the images being acquired during each scan.

All these features make FMD Studio suitable for multi-centre studies on FMD evaluation, when central analysis and/or independent single centre analysis is required.

In conclusion, this study demonstrated a substantial agreement between two different automatic systems for FMD measurement, indicating that clinical studies performed by these methods can be compared without risk of result misinterpretation. The newly proposed system is characterized by important features allowing
a simpler and more reliable evaluation of the FMD which could be of great importance for the future of routinely use of FMD in large clinical trials and for the assessment of cardiovascular risk in the clinical setting.

Therefore, the present work might add an important contribution to the non-invasive assessment of endothelial function.

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References