ULTRASOUND MEASUREMENT OF THE BRACHIAL ARTERY
FLOW-MEDIATED DILATION WITHOUT ECG GATING

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Abstract—The methods commonly used for noninvasive ultrasound assessment of endothelium-dependent flow-mediated dilation (FMD) require an electrocardiogram (ECG) signal to synchronize the measurements with the cardiac cycle. In this article, we present a method for assessing FMD that does not require ECG gating. The approach is based on temporal filtering of the diameter-time curve, which is obtained by means of a B-mode image processing system. The method was tested on 22 healthy volunteers without cardiovascular risk factors. The measurements obtained with the proposed approach were compared with those obtained with ECG gating and with both systolic and end-diastolic measurements. Results showed good agreement between the methods and a higher precision of the new method due to the fact that it is based on a larger number of measurements. Further advantages were also found both in terms of reliability of the measure and simplification of the instrumentation.

Key Words: Flow-mediated dilation, Ultrasound, ECG gating, Image processing.

INTRODUCTION

Flow-mediated dilation (FMD) of the brachial artery is an established noninvasive method used to assess endothelial function (Célermajar et al. 1992). Over the last 15 y, this examination has gained increasing importance since several studies have shown that an impaired FMD response is related to cardiovascular risk factors, such as smoking (Célermajar et al. 1993; Corretti et al. 1998), hypercholesterolemia (Vogel et al. 1996; Toikka et al. 1999), hypertension (Park et al. 2001; Ghidoni et al. 2001), diabetes (Lambert et al. 1996; Kawano et al. 1999) and aging (Célermajar et al. 1994, Ghidoni and Virdis 2006) and is an independent predictor of cardiovascular events (Lerman and Zeiher 2005; Deanfield et al. 2007). The technique most commonly used to assess the FMD is based on ultrasound imaging and was described in the guidelines of the International Brachial Artery Reactivity Task Force (Corretti et al. 2002). The examination consists in measuring the brachial artery diameter at rest and after reactive hyperemia induced by ischemia of the forearm. The measurement is taken on a B-mode section of the artery, which is imaged above the antecubital fossa in the longitudinal plane. In the past, the diameter values were obtained by manual analysis, where the borders of the vessel were identified by means of electronic calipers. More recently, automatic methods have been developed and these have increased the objectivity of the examination and largely reduced the time needed for analysis (Beux et al. 2001; Craiem et al. 2007; Frangi et al. 2003; Gemignani et al. 2007; Newey et al. 2002; Sonka et al. 2002).

In FMD measurements, the timing, with respect to the cardiac cycle, is very important. In fact, vasodilations induced by reactive hyperemia are not much larger than the diameter variations between systole and diastole (Chuang et al. 2002). Guidelines suggest using electrocardiogram (ECG) gating during image acquisition, where the onset of the R-wave is used to identify the end diastole, and this is currently the method most commonly used both for manual and automatic analyses. This requirement, however, influences the complexity of the ultrasound equipment adopted for the examination. Nowadays, high frequency linear array transducers are also available in less expensive hand-carried ultrasound devices, which are being used more and more in research and clinical practice. Although such devices produce
high quality B-mode images, they may lack ECG trigger capabilities, which are at times provided as an option with a significant increase in the overall cost of the system. On the other hand, modern automatic measurement methods used in FMD examinations have become more precise and fast, thus, allowing a continuous measurement of the diameter curve with a sample rate of 25 to 30 samples/s. By using these systems, information on the timing with respect to the cardiac cycle can be obtained by directly analyzing the diameter curve, without the need for an ECG trigger.

In this article, we compared FMD values which were obtained with and without the use of an ECG gating technique. All the measures were obtained by an automatic system we developed in our institute, which is able to provide a precise and continuous measurement of the brachial artery diameter by starting from ultrasound images. Systolic and end-diastolic diameters were retrieved by looking at local maxima and minima, respectively, and these were compared with the diastolic value obtained with the ECG trigger. Furthermore, a new approach based on the temporal filtering of the diameter variation induced by the cardiac cycle is proposed and compared with the previous method both in terms of precision and reliability.

MATERIALS AND METHODS

Twenty-two healthy volunteers without cardiovascular risk factors, 12 males and 10 females, with an age range of 24 to 44 y, were recruited for the study. The study protocol was approved by the local ethics committee and informed consent was obtained from all volunteers. Vascular ultrasound scans were performed in a quiet air conditioned room (20°C to 22°C), with the subjects at rest, supine, with the right arm in the extended position. A B-mode image of the brachial artery was obtained in the longitudinal section between 5 and 10 cm above the elbow, using a linear array 12 MHz transducer and a MyLab 25 (Esaote S.p.A. Genova, Italy) ultrasound system.

The examinations started with a baseline recording of 1 min, a cuff (placed around the forearm just below the elbow) was then inflated for 5 min at 200 mmHg and subsequently deflated to induce reactive hyperemia. The measurement was continued for 3 min subsequent following the cuff deflation.

Image processing system

The measurement of the brachial artery diameter was carried out by an image processing device developed in our institute and currently adopted by a multicenter study for FMD assessment (Gemignani et al. 2007). The system is based on the analysis of B-mode ultrasound images, where the boundaries of the brachial artery are located by a contour tracking technique. Subpixel precision is achieved thanks to both the use of a novel edge detection algorithm and the interpolation of a number of edge points. The analysis is carried out online at 25 diameter samples/s.

The system was also equipped with an ECG sync signal so as to identify the diameter values in coincidence with the onset of the R wave. Therefore, the diameter waveform we obtained in this way is sampled at 25 Hz, it shows the cardiac cycle variations and has markers in coincidence with the ECG sync.

Data analysis

Four diameter measures were obtained from the data collected by the above mentioned system. The ECG-gated diameter value (EDV) was obtained simply by taking the diameter values in coincidence with the ECG syncs. The systolic diameter value (SDV) and the diastolic diameter value (DDV) were calculated from the diameter waveform. To simplify the analysis, however, we used the ECG sync to obtain these values: the SDV and the DDV were obtained by computing, respectively, the maximum and the minimum value of the diameter in a 300 ms interval of the ECG sync.

A fourth measure, which was unaffected by the cardiac cycle was obtained, without the use of the ECG sync, by filtering the diameter waveform. The approach is based on the fact that diameter changes due to vasodilation/vasoconstriction mechanisms and diameter changes induced by the cardiac cycle happen at different frequencies: fractions of Hz for the former; more than 1 Hz for the latter (we suppose the heart rate is greater than 60 beats/min). With this assumption, we can use a low pass filter to separate the two components and obtain only the desired information.

Several solutions can be adopted for temporal filtering. We opted for a finite input response (FIR) filter because this is a solution that can be easily implemented in real-time. The design was carried out by using Matlab (The MathWorks Inc. Natick, MA, USA) and the magnitude response of the filter is shown in Fig. 1. The attenuation at the stopband, that is for frequency greater than 1 Hz, is high enough to provide an effective filtering of the oscillations due to the cardiac cycle. The −6 dB cutoff frequency is 0.3 Hz, which preserves the variations due to vasodilation/vasoconstriction mechanisms. The order of the filter is 100, which guarantees a satisfactory selectivity and generates a delay of 2 s only in the response of the filter, an acceptable value for real-time analysis. In this article, the value obtained by filtering the curve with the FIR filter will be referred to as the filtered diameter value (FDV).

To correctly compare the results obtained with the four methods, a filter was also applied in the first three
measurements. In this case, the data were not sampled at a constant frequency (we have one value every heart beat) so a more complex filtering operation should be applied. However, to simplify the analysis, we used a FIR filter anyway, which was designed for a sample frequency of 1.33 Hz or, in other words, the equivalent of 80 beats/min. The filter has three coefficients and was applied by a simple convolution on the sequence of data, as if these were equispaced samples. The operation is not very different from taking the average of the three measurements coming from three subsequent cardiac cycles, an approach which is commonly used when measuring the diameter for FMD assessment.

Statistical analysis

The diameter values obtained with the four methods were compared in terms of percent dilation

%D = (d – d_{BAS})/d_{BAS} where d_{BAS} is the baseline diameter and d is the diameter at a given instant. For each method, d_{BAS} was computed by averaging the values collected during the first min of examination. Subsequently, the %D was computed at 20 points: it was computed every 10 s in the 10 to 50 s baseline recording interval and every 10 s in the 40 to 180 s interval after the cuff deflation. The sample size was chosen on the basis of previous studies (Gemignani et al. 2007).

Bland Altman plots were used to compare data. In a first analysis, results obtained by the SDV, DDV and FDV were compared with those obtained by the EDV, as the latter is the method most commonly used to measure the FMD. Then the four %D values were averaged to obtain a “gold standard” measure and this was compared with each of the four measures.

RESULTS

Figure 2 shows a portion of a diameter curve from a single subject, where the SDV, DDV and EDV have been highlighted. It can be noticed that there is a certain difference between the ECG trigger and the instant when the diameter reaches the minimum value, which is caused by the pulse wave delay. The average value of such a delay computed over all the examinations was 84 ms. The figure also shows the curve (FDV) obtained after the low pass filter. The residual oscillator is less than 1 μm, which is a negligible value for our analysis.

The FDV also highlighted another artifact, which was present only in some of the examinations we carried out. Some curves (Fig. 3) show cyclic diameter variations at a frequency of few tenths of Hz. The phenomenon is also evident in a frequency analysis of the diameter curve (Fig. 4), where a peak which is far below the

Fig. 1. Magnitude response of the temporal filter.

Fig. 2. Diameter curve and the four diameter measures are showed in a interval of baseline measurement.
cardiac cycle frequency is present. We verified that this artifact is caused by breathing and can be justified by the small movements of the patient’s arm. In fact, we must take into account that in FMD examinations the alignment between the probe and the brachial artery is a particularly critical issue (Gemignani et al. 2007) and even almost imperceptible movements can cause a variation in the measure. This artifact caused by breathing was sometimes not negligible (we found variations in the order of 10 to 20 μm) and cannot be easily filtered because it is at frequencies which coincide with the looked for signal.

Table 1 shows the average value and the standard deviation of the diameter $d$ for the complete set of measurements. The number of data should have been 440 (20 values per 22 examinations) but we took into account only 418 values because 22 points were at intervals where the quality of the images was insufficient. These intervals gave artifacts in the diameter curve which were discharged by the sonographer.

The results of the comparison with respect to the EDV are reported in Fig. 5 and Table 2. A small bias is present in the % FMD measures because of the differences in the diameter measures obtained by the four methods: a larger diameter measure, such as in the SDV and in the FDV, gives smaller percent increments; the opposite happens for the DDV. The SD and, consequently, the interval of agreement, are nearly the same

![Graph showing artifacts](image1)

**Fig. 3.** Portion of curve of the FDV which shows artifacts caused by breathing.

![Graph showing frequency analysis](image2)

**Fig. 4.** Frequency analysis of the diameter curve in a baseline measurement: A peak at 0.1 to 0.2 Hz is present, which is caused by breathing.
for the DDV and FDV, while they are moderately larger for the SDV.

Figure 6 and Table 3 report the results of the comparison with the gold standard measure. The bias, in this case, is negligible for all four measurements. There is a significant difference, however, in the SD, which is higher for the SDV, as we found in the previous analysis and rather lower for the FDV. The better agreement of the FDV can be explained by the wider filtering process of this measure. In fact, while the other three measures are obtained by averaging only three diameter values, the FDV is computed by taking account of an interval of 100 measurements.

Discussion and Summary

This article shows how the FMD can be computed without the need for ECG gating. This is made possible by the fact that modern measurement systems can provide the diameter value with high sample rates (25 samples/s or more). In this case, information on the variation induced by the cardiac cycle can be obtained by directly analyzing the diameter waveform.

All the FMD measures obtained with systolic, diastolic and filtered diameters showed a good agreement with the FMD measure obtained with ECG gated diameter. The agreement was lower when the systolic diameter was used, while it was higher when the filtered diameter was used. The latter also showed the best agreement with a gold standard analysis, which was obtained by averaging the four FMD measures. The best performance of the measure coming from the filtered diameter

Table 1. Mean and standard deviation of the diameter obtained with the four measurement methods

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<th>EDV</th>
<th>SDV</th>
<th>DDV</th>
<th>FDV</th>
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<tr>
<td>Diameter</td>
<td>3.88 ± 0.75</td>
<td>3.92 ± 0.76</td>
<td>3.87 ± 0.74</td>
<td>3.90 ± 0.75</td>
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Table 2. Comparison with respect to the EDV: Results of the Bland Altman analysis

<table>
<thead>
<tr>
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<th>SDV vs. EDV</th>
<th>DDV vs. EDV</th>
<th>FDV vs. EDV</th>
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<tr>
<td>Bias</td>
<td>−0.11%</td>
<td>0.03%</td>
<td>0.02%</td>
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<tr>
<td>SD</td>
<td>0.49%</td>
<td>0.28%</td>
<td>0.24%</td>
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<tr>
<td>Interval of agreement</td>
<td>±0.96%</td>
<td>±0.54%</td>
<td>±0.46%</td>
</tr>
<tr>
<td>95% CI for bias</td>
<td>±0.05%</td>
<td>±0.02%</td>
<td>±0.02%</td>
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<tr>
<td>95% CI for limits of agreement</td>
<td>±0.09%</td>
<td>±0.05%</td>
<td>±0.04%</td>
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Fig. 5. Comparison with respect to the EDV: Bland Altman plots.
can be explained with a higher precision of this kind of data, which is obtained by a filtering operation over a larger number of measurements.

The approach based on filtering also showed other advantages with respect to the other techniques we took into account in this article. As we stated, both the systolic and diastolic diameters can be retrieved from the diameter waveform without the use of an ECG sync. In good quality examinations, this operation is simply obtained by the research of local maxima or minima, respectively. However, in clinical practice, the quality of the images is often suboptimal and the diameter curve has noise or artifacts, which sometimes results in the diameter variation over the cardiac cycle not being easily recognizable. In fact, in these cases, the search for systolic and diastolic diameters may be rather difficult without the use of an ECG sync.

Furthermore, we found that the filtered diameter waveform better highlights artifacts caused by small movements of the patient, such as those induced by breathing. These artifacts cannot be removed easily in post analysis, so they should be somehow prevented. This operation is made easier by the presence of the filtered diameter curve in real-time. In this case, the sonographer can recognize these artifacts before starting the examination and try to modify the position of the patient to minimize the problem.

The absence of an ECG sync is also an advantage both in terms of simplification of the examination and in terms of system requirements. It is important to remember that the assessment of the FMD is still an exam which needs a certain technical skill, so any simplification of the method is important. In the same way, a reduction of the requirements for the ultrasound system helps the diffusion of the technique in the clinical setting.

In summary, there is a growing interest in methods for clinical assessment of endothelial function (Deanfield et al. 2007). This article proposes a method for the

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<th>Table 3. Comparison with respect to the gold-standard measure: Results of the Bland Altman analysis</th>
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<tr>
<td>EDV vs.</td>
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<tr>
<td>GS</td>
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<tr>
<td>Bias</td>
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<td>SD</td>
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<td>Interval of agreement</td>
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<td>95% CI for bias</td>
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<td>95% CI for limits of agreement</td>
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Fig. 6. Comparison with respect to the gold-standard measure: Bland Altman plots.
Flow-mediated dilation of the brachial artery ● V. GEMIGNANI et al. 391

assessment of the FMD that is based on filtering the diameter waveform without the use of an ECG gating technique. The results in terms of vasodilation are not significantly different from those obtained by the more common approach of end-diastolic measurement. The method has been implemented in a system for real-time assessment of the brachial artery diameter obtaining an improvement both in terms of precision of the analysis and in terms of reliability of the exam. The availability of a system, which can be used with less complex and less expensive ultrasound systems, increases the possibility of studying early vascular abnormalities in clinical settings.

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REFERENCES


