Flow-Mediated Slowing as a Methodological Alternative to the Conventional Echo-Tracking Flow-Mediated Dilation Technique for the Evaluation of Endothelial Function: A Preliminary Report

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Abstract

The Moens-Korteweg equation predicts changes in pulse wave velocity (PWV) after changes in arterial radius; therefore, an increase in arterial radius, as seen in a reactive hyperemia (RH) condition, should slow PWV over a given arterial segment. If this assumption is true, then the deceleration of PWV over the brachial artery (flow-mediated slowing [FMS]) should be an equivalent signal of endothelial function during a conventional RH flow-mediated dilation (FMD) procedure. Our aim was to compare FMS with FMD after RH in healthy individuals as part of a study that seeks to evaluate the clinical usefulness of FMS as a noninvasive approach to characterize endothelial function. This cross-sectional study included 25 healthy participants (18 women [72%]) with a mean ± SD age of 21.12 ± 0.73 years. The FMD and FMS were simultaneously measured. A significant correlation was observed between both measures of FMS (absolute difference and percentage variation) and echo FMD: $r = 0.42$ ($P = .04$) and $r = 0.46$ ($P = .02$), respectively. The FMS was shown to depend on the baseline brachial diameter, with smaller variations depicted for smaller baseline brachial diameters. It seems to be a promising and feasible method for measuring changes after RH, although further studies are needed to evaluate how this correlation holds in different clinical conditions and to demonstrate its clinical usefulness.

The role of endothelial dysfunction (ED) in the natural history of cardiovascular disease is well-known and is acknowledged as one of the earliest stages in the physiopathologic continuum of atherosclerosis. Thus, ED is an important cardiovascular risk marker, with significant prognostic implications. To date, flow-mediated dilation (FMD) is recognized as the reference method for assessing endothelial function (EF) noninvasively. The percentage increase in brachial artery diameter after reactive hyperemia (RH) defines the FMD and relates to the endothelium’s ability to release and respond to nitric oxide, thus indicating its functional health. Although this technique has strong advantages, it also has some important disadvantages, such as being highly operator-dependent, which could determine unacceptable variability unless a dedicated technician and adequate equipment are present. As an alternative to the conventional FMD technique, pulse wave velocity (PWV) could offer a much easier and more reproducible methodological alternative to studying EF. In fact, the Moens-Korteweg equation links PWV to the arterial radius, as follows: \[ PWV = \sqrt{\frac{E \cdot h}{D \cdot \delta}} \] where $E$ is the elastic modulus; $h$, the wall thickness; $D$, the arterial diameter; and $\delta$, the blood density. The theoretical consequence of this is that if the arterial radius $R$ increases after RH, a decrease in PWV is expected to occur. If this assumption is true, then the deceleration of PWV over the brachial artery (what we designate as flow-mediated slowing [FMS]) should be an equivalent signal of EF during a conventional RH procedure. Because PWV is a validated
method, easy to perform, highly reproducible, and low cost, the demonstration of its usefulness for evaluating ED should be of major interest. To date, just a few studies have explored this hypothesis.

Based on this premise, we present the preliminary results of an ongoing project that aims to appraise the clinical usefulness of FMS for the evaluation of ED, particularly focusing on the comparison between FMS and FMD after RH in healthy individuals.

METHODS

We conducted a cross-sectional study aimed at comparing FMS and FMD estimations of EF in 25 clinically healthy participants (18 women [72%]) with a mean ± SD age of 21.12±0.73 years.

Evaluations were conducted in a laboratory with controlled luminosity, temperature, and humidity. All the evaluations took place in the morning, with participants fasting and deprived from smoking, exercise, alcohol, and caffeine for 12 hours before the study. Demographic data were collected for each participant. The participants were then placed in the supine position, and after a 10-minute rest period, brachial blood pressure was measured using an automatic and clinically validated sphygmomanometer (ri-champion N; Rudolf Riester GmbH) with a cuff that adjusted for the arm diameter. Afterward, FMD was obtained, complying with the methodological recommendations.8,12 A Vivid 3 echograph (General Electric) equipped with a linear probe (frequency range, 7-12 MHz) was used. A basal measurement of the diameter of the right brachial artery in a linear plane was made, approximately 2 to 3 cm off the antecubital fossa. Baseline carotid-radial PWV was then acquired in the same arm using the Complior Analyse device (Alam Medical). For this, a probe with piezoelectric crystals was placed on the right radial artery and another on the ipsilateral carotid artery. The probes were adjusted to ensure the acquisition of pulse waves with suitable reproducibility, stability, and amplitude. The distance between the 2 points was measured directly. The brachial PWV corresponded to the distance (d) divided by the pulse wave transit time (PTT) between the 2 arterial territories considered (carotid-radial), where PWV = d/PTT (m/s).

Subsequently, a cuff was placed on the forearm, distal to the site of the ultrasound assessment, and was inflated into a suprasystolic pressure (~ 50 mm Hg above the previously measured systolic blood pressure), keeping the ischemia for a 5-minute period, after which the cuff was deflated. Approximately 1 minute after complete deflation of the cuff, the diameter of the brachial artery and the brachial PWV were simultaneously measured. The FMD was calculated as the percentage increase in brachial diameter after RH. The FMS was calculated using the baseline PWV and the PWV after RH, as ∆PWV, quantifying the absolute difference between the 2 moments of PWV, and as %PWV, quantifying the percentage of variation. All measurements were performed in the right arm.

All the tests were performed by the same experienced operator (T.P.) to ensure the necessary reproducibility conditions. All the
participants agreed voluntarily to participate in the study and gave their informed consent.

IBM SPSS Statistics for Windows, version 23.0 (IBM Corp), was used. Simple descriptive statistic was used to characterize the population. Data are presented as mean ± SD for continuous variables and as absolute frequency (percentage) for categorical variables. Continuous variables were compared using the independent t test, and categorical variables were compared using the Fisher exact test. The correlations between continuous variables were determined using the Pearson R correlation test. A \( P < .05 \) was set as the criterion for statistical significance for a 95% CI.

RESULTS

The Table presents the characteristics of the population (N=25), with a mean ± SD age of 21.12±0.73 years and a mean ± SD body mass index (calculated as the weight in kilograms divided by the height in meters squared) of 22.10±3.19. Men (n=7) were taller and heavier, had higher systolic and pulse pressures, and had greater baseline brachial diameters. No participant had cardiovascular risk factors or a history of cardiovascular disease. No participant was under any long-term medication intake, except for the use of oral contraceptives, seen in most of the female participants.

Regarding the relationship between the estimates of EF derived from the conventional FMD technique and those from the innovative FMS procedure, significant correlation coefficients were obtained by bivariate correlation analysis (Figure), even when adjusting for potential confounding variables, thus indicating a significant and moderate correlation between FMD and \( \Delta \text{PWV} \) (\( R = -0.42; P = .04 \)) and between FMD and \%PWV (\( R = 0.46; P = .02 \)). As depicted in the Figure, a greater PWV slowing effect was depicted for participants showing a greater increase in the brachial diameter after RH, according to the predictions of the Moens-Korteweg equation.9

An additional cluster analysis was performed to explore individual differences, and 2 main groups of participants were extracted in terms of the consistency in the estimates of EF with the comparing methods: group 1 comprised participants with a consistent relationship between FMD and FMS, and group 2 comprised participants with little concordant or even discordant results. Based on this definition, significant differences were identified mainly in the basal brachial diameters between the 2 groups (\( P = .02 \)), implying

| \( \Delta \text{PWV (ms)} \) | \%PWV (%) |
|---------------------|-----------|
| .0                   | 30.00     |
| –2.50               | –10.00    |
| –2.00               | 20.00     |
| –1.50               | 5.0       |
| –1.00               | 0.0       |
| –.50                |           |
| 0.0                 |           |
| 5.0                 | 10.0      |
| 10.0                | 15.0      |
| 15.0                | 20.0      |
| 20.0                |           |

FIGURE. Relationship of pulse wave velocity (PWV) absolute difference (\( \Delta \text{PWV} \)) and PWV percentage variation (\%PWV) with flow-mediated dilation (FMD).
that the correlation between the 2 methods seems to be diameter dependent so that FMS tends to be lower in individuals with smaller baseline arteries.

**DISCUSSION**

The importance of ED has been widely established as a starting point in the continuum of the most significant cardiovascular diseases. Therefore, efforts to develop accurate noninvasive methods for use in clinical practice are of utmost importance. Currently, the echo-based FMD procedure is widely acknowledged as the reference method for this purpose, although some important limitations are identified, chiefly in terms of reproducibility, cost, and expertise requirements.6,10

Simpler, cheaper, and more reproducible alternatives should, thus, be considered, and the proposed concept of FMS could be an interesting hypothesis to explore. These preliminary results pinpoint a significant correlation between FMD and FMS in a healthy and young population. In fact, larger FMD values were followed by a greater deceleration of the pulse wave in the carotid-radial area, as would be expected from the knowledge that the bigger the vessel, the lower the resistance, and, therefore, less will be the velocity, and also following the predictions in the Moens-Korteweg equation, by which PWV is directly proportional to the arterial wall thickness and its elastic modulus and is inversely proportional to vessel diameter and blood viscosity.11,13 Curiously, women had smaller baseline brachial artery diameters, in line with previous research,4,14 but showed a trend for lower baseline brachial PWV compared with men, pinpointing the influence of other physiologic regulation vectors as relevant modulators for propagation of the pulse wave, from which the elastic modulus may play a quite important role.11 Similar results were previously reported15 comparing the brachial PWV response with different durations of ischemia, demonstrating greater reductions in PWV to increasing ischemia durations, peaking at 5 minutes of RH.

The results obtained should be envisioned as a preliminary attempt to develop a new concept of noninvasively studying EF, considering the limitations of the study, mainly the small population included (N=25) and the particular clinical features of the participants. The replication of this study in a larger population, including a wider spectrum of clinical subsets, with participants with known risk factors,15 and in a longitudinal design would provide further insight into the usefulness of this method for identifying ED and discriminating cardiovascular risk profiles.

In conclusion, FMS seems to be a promising indirect method for the assessment of ED, although further research is needed to better characterize its validity and clinical usefulness.

**Abbreviations and Acronyms:** EF = endothelial function; FMD = flow-mediated dilation; FMS = flow-mediated slowing; PWV = pulse wave velocity; RH = reactive hyperemia.

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