Measurement of Flow-Mediated Vasodilatation
A Lesson From the FMDJ Study

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Summary
The FMDJ study, a multicenter prospective observational study conducted in Japan, demonstrated the acceptable reliability of measurement of flow-mediated vasodilatation (FMD) using a semi-automatic device in individual institutions. However, in about 10% of Japanese subjects, adequate scans to determine the brachial arterial diameter failed to be obtained. The prevalence of inadequate scans was higher in women than in men, while obesity had no influence on the inadequate scan rate. The FMDJ study also proposed that attending periodic refresher courses on the measurement of FMD is needed for maintaining competency. Finally, the FMDJ study proposed reference values for FMD. Thus, FMD measurement may be categorized as a clinically applicable tool on the basis of class IIb (exploratory cohort study with good reference standards) evidence. (Int Heart J 2017; 58: 158-162)

Key words: Endothelial function, Reproducibility, Reference value

Endothelial dysfunction is the initial step of atherosclerotic vascular damage, and is known to have pivotal roles in the initiation/progression of atherosclerosis. Flow-mediated vasodilatation of the brachial artery (FMD) is a well-recognized marker of endothelial function, and several studies have suggested the usefulness of FMD measurement in risk stratification for cardiovascular disease at all stages of the cardiovascular disease continuum. Nonetheless, FMD measurement is still not clearly recommended as a useful screening tool for primary and secondary prevention of cardiovascular disease. The reasons for this narrow application of FMD measurement as a clinical tool are as follows: 1) FMD measurement requires significant technical expertise; 2) no standard protocol (eg, the inflation pressure needed to cause ischaemia, the optimal duration of ischaemia, brachial versus wrist cuff inflation, and the time point at which the effect of reactive hyperemia still need to be precisely assessed) is available for the measurement of FMD; and 3) there are no reference values for FMD.

Recently, an ultrasound instrument dedicated to FMD assessment, equipped with both an on-line computer-assisted semi-automatic analysis software to measure the FMD and accessories for fixing the measured arm and ultrasound probe, was introduced for commercial use in Japan. A major advantage of this device is that it allows automatic direct A-mode signal analysis of changes of the vessel diameter during the FMD assessment procedure in real time under stereotaxic guidance based on 2-dimensional B-mode images for maintenance of the appropriate position for recording of the vessel interfaces. Thus, this device might be helpful for measurement of FMD even by persons with lower levels of technical expertise and to establish a standard protocol for the measurement. The FMDJ study is a multicenter prospective study whose aim was examining the usefulness of FMD measurement by an online semi-automatic software in the risk stratification for cardiovascular diseases in Japanese subjects; FMD data of more than 8,000 subjects were recorded in the FMDJ study. Based on the findings of the FMDJ study, we provide a brief review herein of how to measure FMD and of the applicability of measurement of FMD as a clinical tool.

Standardization of FMD Measurement
The protocol used to measure FMD in the FMDJ study is summarized in Table 1. In this protocol, “An occlusion cuff is wrapped around the forearm with the proximal edge of the cuff at the level of the elbow”. While wrapping the cuff around the upper arm allows a greater degree of dilatation of the brachial artery after release of the cuff pressure, wrapping it around the forearm allows easy recording of the brachial artery by ultrasound and also easy maintenance of inter-institutional generalizability of the method. In the FMDJ study, the time needed to obtain images of the brachial artery after release of the cuff pressure varied from institution to institution (range, 5 to 30 seconds); therefore, the diameter measured before cuff compression was used as the baseline diameter.

Technical Expertise and Reliability of FMD Measurement
Technical aspects are important determinants of the reproducibility of FMD measurement. For acceptable reliability of FMD measurements in individual institutions, training of
All the sonographers who performed the FMD measurements in the FMDJ study visited the core laboratory designated by the study at Tokyo Medical University to receive training on a standard protocol for the measurement and on how to scan and analyze the scan records using the manual (https://ncsg.jp/fmdj/). Recently, a regular online seminar on how to measure FMD has become available for cardiovascular technicians (http://plaza.umin.ac.jp/~cvt/updating.html); a video on how to measure FMD is also now available online (https://ncsg.jp/fmdj/course.jsp).

As we have already reported, a major objective of the FMDJ study was to validate the reliability of FMD assessment (scanning and analysis of the scans) using a semi-automatic device at individual participant institutions as compared to analysis of the brachial artery scans at a core laboratory (Figure 1). The results of assessment of the quality of the brachial artery scans and of the data analyses conducted at each institution were registered on the website of the study. Each participant institution (18 institutions) was instructed to send USB drives containing the brachial artery scans recorded for the assessment of FMD, but not the results of the data analysis, to the core laboratory located in Tokyo Medical University.

The findings of this validation study are briefly summarized as follows: A total of 981 subjects were registered in this study. Inadequate scans were examined to check which of the following two criteria for inadequate scans they met: exclusion criterion 1 = lack of clarity of the vessel interfaces in the longitudinal B-mode images and/or inadequate images obtained due to patient movement (n = 101) (Figure 2); exclusion criterion 2 = exclusion criteria 1 plus an unclear signal of the intima-media complex (IMC) in the A-mode images (n = 66). After the scans which fulfilled exclusion criterion 1 were excluded (n = 880) (Figure 3), the correlation coefficient of the FMD values measured between the core laboratory and each institution improved from $r = 0.725$ to $r = 0.838$. On the other hand, exclusion of scans that fulfilled criterion 2 (ie, absence of a clear image of the peak IMC signal in A-mode images) did not affect the correlation coefficient of the FMD values measured between the core laboratory and each institution (n = 814, $r = 0.840$). Thus, when the analysis was limited to the scans which fulfilled exclusion criterion 1, the reliability of the FMD assessment conducted in individual institutions appeared to be acceptable.

The rejection rate of scans on the basis of exclusion criterion 1 was around 10% in Japanese subjects. It is therefore necessary to recognize that in its present state, this FMD assessment method may not be applicable to all subjects. In the study subjects of the FMDJ study (n = 8363), we attempted to identify the characteristic clinical features of the subjects with inadequate scans by logistic regression analysis, and identified non-smoking status, female gender, and young age as sig-
significant clinical features associated with inadequate scans (Table II). Interestingly, while the BMI was over 30 in 337 of the subjects enrolled in the study, obesity (BMI ≥ 30) was not associated with inadequate scans. Based on the findings, it is important to take into account the gender difference in the prevalence of inadequate scans (inadequate scan in men = 694/6742 (10%), inadequate scan in women = 339/1909 (18%)).

As described in Table I, the arterial diameter is determined by the peak signals of the IMC in A-mode images. It is important to identify whether the scans are adequate for determining the arterial diameter in the longitudinal view, because if not, the IMC signal cannot be identified (Figure 4). Of the subjects with scans not fulfilling exclusion criterion 1 (n = 880), 66 subjects had scans that fulfilled exclusion criterion 2, and 45 of these latter subjects (69%) had inadequate scans in respect of both the far-wall and near-wall IMC signal. Thus,

Table II. Results of Logistic Regression Analysis to Identify the Clinical Features of the Subjects With Inadequate Scans

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds</th>
<th>Standard error</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>1.817</td>
<td>1.559 - 2.117</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.976</td>
<td>0.969 - 0.983</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoke</td>
<td>0.795</td>
<td>0.704 - 0.898</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Obese30</td>
<td>0.922</td>
<td>0.785 - 1.082</td>
<td>0.319</td>
</tr>
<tr>
<td>MEDdm</td>
<td>0.791</td>
<td>0.562 - 1.115</td>
<td>0.181</td>
</tr>
<tr>
<td>MEDlipid</td>
<td>0.993</td>
<td>0.781 - 1.262</td>
<td>0.953</td>
</tr>
<tr>
<td>MEDhbp</td>
<td>0.881</td>
<td>0.722 - 1.076</td>
<td>0.214</td>
</tr>
</tbody>
</table>

Gender: 1 indicates men and 2, women; age: every decade from 20 to 60; smoke: 0, non-smoker and 1, current smoker; obese: 0, body mass index < 30 and 1, ≥ 30; MEDdm: 0, non-medication and 1, medication for diabetes mellitus; MEDlipid: 0, non-medication and 1, medication for dyslipidemia; and MEDhbp: 0, non-medication and 1, medication for hypertension.
even among subjects with clear vessel interfaces in the longitudinal B-mode images, some (45/880 subjects; 5%) failed to show a clear peak IMC signal in the A-mode images. These finding must be taken into account while attempting to identify appropriate scans for measuring the arterial diameter. In cases without a clear signal of the IMC on the scans, stereotaxic guidance based on 2-dimensional B-mode images may be useful to determine the appropriate diameter.

**Maintenance of Competency**

The guidelines propose that the technician needs a minimum of 100 supervised scannings before he/she can carry out the scanning independently, and scanning of at least 100 subjects/year to maintain competency. In the FMDJ study, the intra-class correlation coefficient (ICC) between each institute and the core laboratory was calculated for assessing the inter-institution generalizability. Among the 5 institutions in which less than 20 subjects were registered in the FMDJ study, the ICC was not significant in 3 institutions. Figure 4 shows the correlations of the FMD values measured at the institution and those measured at the core laboratory for institutions with less or more than 20 subjects registered in the FMDJ study; the correlation coefficient differed between the two groups ($r = 0.048$ versus $r = 0.686$, Figure 5). Thus, after receiving initial training for the assessment of FMD using the semi-automatic device, attending periodic refresher courses in FMD assessment is necessary for maintaining competency. However, we could not clarify the number of scans needed to maintain competency.

**Reference Value of FMD**

The FMDJ study has already reported age-based reference values of FMD based on examination of 4533 subjects, after exclusion of subjects over the age of 75 years and subjects with cardiovascular risk factors. Figure 6 shows the age-based reference values in men and women. The slope of the age-related decline of FMD was steep in subjects < 50 years of age, in both men and women, becoming more gradual in subjects ≥ 50 years; the values were located around 6% in both genders. As our personal opinion, while age and gender differ-

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**Figure 4.** Appropriate and inappropriate scans of the brachial artery.

**Figure 5.** Correlations between flow-mediated dilatation of the brachial artery analyzed at each participant institution and at the core laboratory for participant institutions with more than 20 subjects and those with less than 20 subjects registered in the FMDJ study. FMD indicates flow-mediated vasodilatation of the brachial artery.
ences in FMD values must be taken into account in subjects under 50 years of age, it appears that FMD = 6% can be simply applied as a reference value in Japanese men and women aged ≥ 50 years.

Next Step

Based on the findings of the FMDJ study (ie, establishment of a standard protocol, confirmation of the generalizability of the FMD assessment method, and establishment of reference values), evidence for establishment of FMD measurement as a clinical tool may have risen a step up from class IIIb (without consistently applied reference standards) to class IIb (exploratory cohort study with good reference standards). As the next step, the usefulness of FMD assessment as a marker for the management of cardiovascular disease and/or its risk factors (ie, to clarify whether improvement of FMD by intervention(s) might yield improvement of the cardiovascular prognosis) needs to be clarified.

REFERENCES


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