Borderline Ankle-Brachial Index Value of 0.91–0.99 Is Associated With Endothelial Dysfunction

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Background: An ankle-brachial index (ABI) value of 0.91–0.99 is considered borderline and associated with an increased risk of cardiovascular events. However, there is no information on the relationship between borderline ABI and endothelial function.

Methods and Results: We measured ABI and assessed vascular function by flow-mediated vasodilation (FMD) and nitroglycerin-induced vasodilation in 389 subjects who underwent health examinations. Subjects were divided into 3 groups according to ABI (normal group: 1.00–1.40, borderline group: 0.91–0.99, abnormal group: ≤0.90 or >1.40). FMD was significantly smaller in both the borderline and the abnormal group than in the normal group. There was no significant difference in the vascular responses to nitroglycerin between the normal and borderline groups. Vascular response to nitroglycerin was significantly higher in the normal group than in the abnormal group. Borderline and abnormal ABI values were significantly associated with an increased odds ratio of low tertile of FMD levels, using the normal ABI group as the reference. Multiple logistic regression analysis for FMD revealed that age, sex, hypertension, diabetes mellitus, and borderline ABI independently remained associated with FMD.

Conclusions: ABI of 0.91–0.99 is associated with endothelial dysfunction. ABI examination is a simple and cost-effective method for obtaining the additional information on the initial step of atherosclerosis beyond the assessment of peripheral artery disease. (Circ J 2014; 78: 1740–1745)

Key Words: Ankle-brachial index; Endothelial function; Intima-media thickness; Peripheral artery disease; Vascular smooth muscle function
normal ABI. 24

Recently, several investigators have reported that an ABI value of 0.91–0.99 should be considered borderline and that it is associated with an increasing risk of cardiovascular disease. 25–26 However, there is no information on the association between a borderline ABI value and vascular function. Therefore, the purpose of this study was to evaluate the relationship between ABI value and vascular function in a general population of subjects who underwent health examinations, including patients with cardiovascular diseases.

Methods

Subjects

Between September 2007 and June 2013, a total of 389 subjects (mean age, 58±18 years), including patients with cardiovascular diseases, who underwent health examinations at Hiroshima University Hospital were enrolled in this study. Hypertension was defined as systolic blood pressure (SBP) >140 mmHg or diastolic blood pressure >90 mmHg while seated on at least 3 different occasions. Diabetes mellitus was defined according to the American Diabetes Association. 27 Dyslipidemia was defined according to the third report of the National Cholesterol Education Program. 28 The institutional ethical committees approved the study protocol and written informed consent for participation in the study was given by all subjects.

Study Protocol

We measured ABI and assessed vascular function by measurement of FMD and nitroglycerin-induced vasodilatation in all subjects. We categorized subjects into 3 groups according to ABI (normal group: ABI 1.00–1.40; borderline group: ABI 0.91–0.99; abnormal group: ABI ≤ 0.90 or >1.40).

Subjects fasted overnight for at least 12 h and the study began at 08:30 hours. The subjects remained supine in a quiet, dark, air-conditioned room (constant temperature of 22–25°C) throughout the study. A 23-gauge polyethylene catheter was inserted into the left deep antecubital vein to obtain blood samples. At 30 min of maintaining a supine position, FMD and nitroglycerin-induced vasodilation were measured. The observers were blind to the form of examination.

Measurement of ABI

The subjects remained supine for at least 5 min and cuffs were wrapped around both brachia and ankles. An oscillometric method was used to measure SBP in the bilateral brachial and posterior tibial arteries (form PWV/ABI, Colin Co, Tokyo, Japan). The ABI value was calculated by dividing the highest pressure in the posterior tibial arteries on the right and left sides by the highest brachial pressure on either side. For the purposes of this analysis, the lower of the 2 ABI values was taken as the ABI measurement.

Measurement of Brachial Intima-Media Thickness (IMT)

Before FMD measurement, baseline longitudinal ultrasonographic images of the brachial artery, obtained at the end of diastole (defined as the R wave of the ECG) with a linear, phased-array high-frequency (10-MHz) transducer using an UNEXEF18G ultrasound unit (UNEX Co, Nagoya, Japan) from each of 10 cardiac cycles, were automatically stored on a hard disk for off-line assessment of IMT. 29 Measurement of IMT was automatically performed on A-mode images of the far wall of the brachial artery. The analysis system automatically chose the measurement point where an image of the posterior intimal interface was clearly obtained. If the measurement point was apparently inappropriate, another clear image site could be manually selected for measurement. A total of 21 points over a 3-mm length of IMT in the 10-mm longitudinal image depicted on the analysis display were measured and the mean value per image was automatically calculated. IMT was measured at the same point in each different image. The average of mean values obtained from 10 cardiac cycles was defined as the IMT of the brachial artery. When measuring carotid IMT, we had an anatomical landmark, such as the carotid-artery bulb. Unfortunately, it is difficult to measure the same site in the brachial artery because of the lack of an anatomical landmark. Measurement of IMT in the brachial artery was performed at the site where the clearest B-mode image of the anterior and posterior intimal interfaces between the lumen and vessel wall was obtained at 5–10 cm above the elbow. There was little influence of intra- and inter-patient variability in the measurement site because the interface of the intima-media of brachial artery is relatively smooth and IMT was not localized nor were plaques present, resulting in diffuse IMT.

Measurement of FMD

The subjects remained supine throughout the FMD study. The vascular response to reactive hyperemia in the brachial artery was used for assessment of

| Table 1. Clinical Characteristics of the Subjects on the Basis of ABI |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variable        | Total (n=389)   | Normal (n=287)  | Borderline (n=35) | Abnormal (n=67) |
| Age, years      | 58±18           | 56±18           | 62±16            | 66±13           | <0.001         |
| Sex, M/F        | 261/128         | 190/97          | 21/14            | 50/17           | 0.26           |
| BMI, kg/m²      | 23.3±3.9        | 23.5±3.9        | 23.2±4.0         | 22.3±3.8        | 0.04           |
| Hypertension, n (%) | 259 (66.6)     | 184 (64.1)      | 23 (65.7)        | 52 (77.6)       | 0.09           |
| Dyslipidemia, n (%) | 221 (56.8)     | 156 (54.4)      | 24 (68.6)        | 41 (61.2)       | 0.19           |
| Diabetes mellitus, n (%) | 127 (32.7)     | 76 (26.5)       | 17 (48.6)        | 34 (50.8)       | <0.001         |
| CAD, n (%)      | 73 (18.8)       | 46 (16.0)       | 9 (25.7)         | 18 (26.9)       | 0.07           |
| Stroke, n (%)   | 27 (6.9)        | 8 (2.8)         | 3 (8.6)          | 16 (23.9)       | <0.001         |
| Smoker, n (%)   | 107 (27.5)      | 84 (29.3)       | 6 (17.1)         | 17 (25.4)       | 0.26           |
| Framingham risk score | 5.5±5.2 | 4.9±5.3         | 6.6±5.5          | 7.4±3.5         | 0.002         |
| FMD, %          | 4.6±2.8         | 5.1±2.7         | 3.6±1.9          | 3.1±2.8         | <0.001         |
| Nitroglycerin-induced vasodilation, % | 12.4±6.0 | 13.3±5.7 | 11.2±4.8 | 9.0±6.4 | <0.001 |
| Brachial artery IMT, mm | 0.33±0.08 | 0.32±0.08 | 0.32±0.07 | 0.36±0.08 | 0.004 |

All results are presented as mean±SD. 
ABI, ankle-brachial index; BMI, body mass index; CAD, coronary artery disease; FMD, flow-mediated vasodilation; IMT, intima-media thickness.
es over the cardiac cycle was displayed in real time using the FMD mode of the tracking system. This allowed the ultrasound images to be optimized at the start of the scan and the transducer position to be adjusted immediately for optimal tracking performance throughout the scan. Pulsed Doppler flow was assessed at baseline and during peak hyperemic flow, which was confirmed to occur within 15 s after cuff deflation. Blood flow velocity was calculated from the Doppler data and displayed as a waveform in real time. The baseline longitudinal image of the artery was acquired for 30 s, and then the blood pressure cuff was inflated to 50 mmHg above systolic pressure for 5 min. The longitudinal image of the artery was recorded continuously until 5 min after cuff deflation. Pulsed Doppler velocity signals were obtained for 20 s at baseline and for 10 s immediately after cuff deflation. Changes in brachial artery diameter were immediately expressed as a percentage change relative to the vessel diameter before cuff inflation. FMD was automatically calculated as the percentage change in peak vessel diameter from the baseline value. Percentage of FMD (peak diameter-baseline diameter/baseline diameter) was used for analysis. Blood flow volume was calculated by multiplying the Doppler flow velocity (corrected for the angle) by heart rate and vessel cross-sectional area (-r2). Reactive hyperemia was calculated as the maximum percentage increase in flow after cuff deflation compared with baseline flow.

Assessment of Endothelium-Independent Vasodilation

The response to nitroglycerin was used for this examination. After acquiring a baseline resting image for 30 s, a sublingual tablet (nitroglycerin 75 μg) was given and the image of the artery was recorded continuously for 5 min. Nitroglycerin-induced vasodilation was automatically calculated as a percentage change in peak vessel diameter from the baseline value. Nitroglycerin percentage (peak diameter-baseline diameter/baseline diameter) was used for analysis.

Statistical Analysis

Results are presented as means±SD for continuous variables and percent (%) for categorical variables. Statistical significance was set at P<0.05. Comparisons between groups were carried out using P for trend analysis. Unpaired Student’s t-test was used for group comparisons. Crude and adjusted odds ratios of low tertile of FMD (<3.2%) according to ABI value were calculated by using the normal ABI group as the reference. In subjects with ABI 0.91–1.40, stepwise multiple regression analysis was performed to find independent predictors of FMD among potential confounders (P<0.2) in the univariate analysis. Using variables associated with the FMD in the stepwise multiple regression analyses, we performed the final logistic regression analyses for which results are presented. The data were processed using the software package Stata version 9 (Stata Co, College Station, TX, USA).

Results

Baseline Clinical Characteristics

The baseline characteristics of the 389 subjects are summarized in Table 1. Of the 389 subjects, 261 (67.1%) were men and 128 (32.9%) were women; 259 (66.8%) had hypertension, 221 (56.8%) had dyslipidemia, 127 (32.7%) had diabetes mellitus, and 107 (27.5%) were current smokers. Of the 389 subjects who were evaluated, 73 (18.8%) had known coronary artery disease and 27 (6.9%) had known history of stroke.

Relationships Between Vascular Function and ABI

We divided the subjects into 3 groups according to ABI.
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Table 2. ORs and 95% CIs for Low Tertile of FMD According to ABI

<table>
<thead>
<tr>
<th>ABI</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
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<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>1.00–1.40</td>
<td>1 (reference)</td>
<td></td>
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<tr>
<td>0.91–0.99</td>
<td>2.67 (1.30–5.47)</td>
<td>0.008</td>
</tr>
<tr>
<td>≤0.9 or &gt;1.40</td>
<td>3.94 (2.28–6.89)</td>
<td>&lt;0.001</td>
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Abbreviations as in Table 1.

Low tertile of FMD is less than 3.2%. *Adjusted for age and sex.

ABI, ankle-brachial index; CI, confidence interval; OR, odds ratio.

Borderline ABI and Endothelial Function

(Table 1). There were significant differences between the groups in age, body mass index, diastolic blood pressure, heart rate, prevalence of diabetes mellitus, previous stroke, Framingham risk score, FMD, nitroglycerin-induced vasodilation and brachial IMT. FMD were significantly smaller in the borderline and abnormal groups than in the normal group (3.6±1.9% and 3.1±2.8% vs. 5.1±2.7%, P<0.003 and P<0.001, respectively). There was no significant difference in FMD between the borderline and abnormal groups (Figure A). Vascular response to nitroglycerin was significantly smaller in the abnormal group than in the normal group (9.0±6.4% vs. 13.3±5.7%, P<0.001). There were no significant differences in the vascular responses to nitroglycerin between the normal and borderline groups or between the borderline and abnormal groups (Figure B).

IMT of the brachial artery was significantly greater in the abnormal group than in the normal and borderline groups (0.36±0.08 mm vs. 0.32±0.08 mm and 0.32±0.07 mm, P=0.001 and P=0.03, respectively), but there was no significant difference in brachial IMT between the borderline and abnormal groups (Figure C).

Table 2 shows the crude and multivariate-adjusted odds ratios of the low tertile of FMD according to ABI value. After adjustment for age and sex, both a borderline and an abnormal ABI were significantly associated with an increased odds ratio of low tertile of FMD levels, using the normal ABI group as the reference. Multiple logistic regression analysis for FMD revealed that age, sex, hypertension, diabetes mellitus, and borderline ABI independently remained associated with FMD (Table 3).

Discussion

In the present study, we demonstrated that a borderline ABI is associated with endothelial dysfunction but not endothelium-independent vasodilation, suggesting that even subjects with borderline ABI have prior impaired endothelial function, even though vascular smooth muscle function is maintained in these subjects. These harmful effects on the vasculature may contribute to the increased prevalence of cardiovascular events in subjects with borderline ABI compared with subjects with a normal ABI. The ABI, especially a borderline value, is useful in clinical practice for assessing premature atherosclerosis that leads to cardiovascular events. To our knowledge, this is the first report showing an association of borderline ABI with endothelial dysfunction.

Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis. In addition, impaired endothelial function is proportional to the severity of atherosclerosis. Previous studies have clearly shown that endothelial function is a predictor of cardiovascular events. Interestingly, in the present subjects with borderline ABI, FMD was impaired, while nitroglycerin-induced vasodilation and brachial IMT were maintained in the normal ranges. In the subjects with abnormal ABI, FMD and nitroglycerin-induced vasodilation were impaired, and brachial IMT was increased. These findings suggest that although subjects with an abnormal ABI have endothelial dysfunction and abnormal vascular structure, those with a borderline ABI still have normal vascular structure. In subjects with an abnormal ABI, increased brachial IMT may be related to an increase in connective tissue matrix and proliferation of vascular smooth muscle cells, leading to an impaired vasodilatory response to nitroglycerin. Several investigators, including us, have shown that patients with severe atherosclerosis may have irreversible impairment of endothelial function. It is clinically important that treatment of atherosclerosis should be appropriately undertaken before the development of advanced atherosclerosis with severely damaged blood vessels so as to prevent cardiovascular events. Our findings emphasize the clinical importance of instituting adequate interventions to prevent the development of atherosclerosis. We should pay attention to borderline ABI values as an indicator of early-stage atherosclerosis and also endothelial dysfunction.

It is thought that the borderline ABI value of 0.91–0.99 means moderate plaque progression in lower limb arteries. We previously reported that vascular function is different in the arteries of the upper and lower extremities in patients with PAD. In the present study, we found that subjects with a borderline ABI still had normal vascular structure in the brachial artery. These findings suggest that atherosclerosis progresses earlier in the lower limb arteries than in the upper limb arteries. Although the precise mechanism for the difference in vascular function remains unclear, possible reasons are differences in blood pressure and exercise quantity between the upper limb and lower limbs.

It is well known that hypertension, dyslipidemia, diabetes, aging, smoking, and obesity are contributing risk factors in cardiovascular and cerebrovascular diseases. These risk factors are also independent predictors of endothelial dysfunction. Sev-
eral lines of evidence have shown the prevalence of cardiovascular disease and cardiovascular events increases in relation to a decrease in ABI of <0.9, including borderline values of ABI.\textsuperscript{17–20} In addition, PAD or abnormal ABI is associated with endothelial dysfunction.\textsuperscript{14} In the present study, there were significant differences in age, body mass index, and presence of diabetes mellitus and stroke, as well as Framingham risk score, which is a risk calculator and an index of cumulative cardiovascular risk commonly used for assessing the probability of heart attack or death from heart disease within 10 years according to the ABI. Our multivariate regression analysis revealed that a borderline ABI remained an independent predictor of FMD. ABI, especially a borderline ABI, is a good marker of not only the early stage of atherosclerosis but also endothelial function under the condition of the presence of cardiovascular risk factors. Measurement of ABI provides additional information on the initial step in the pathogenesis of atherosclerosis beyond diagnosis and assessment of the severity of PAD.

**Study Limitations**

In general, high ABI (>1.40) is related to calcification of the artery, which may occur commonly in patients with advanced atherosclerosis, including patients with severe diabetes mellitus and patients with end-stage renal disease.\textsuperscript{21,22} Several studies have shown that a high ABI as well as a low ABI is associated with future cardiovascular events.\textsuperscript{23–25} Both FMD and nitroglycerin-induced vasodilation were lower in subjects with high ABI values than in subjects with normal ABI (2.6±0.9% and 6.4±4.0%, P<0.001, respectively). However, in the present study, there were only 8 subjects (2.0%) with a high ABI, which was too small for statistical analysis. Therefore, these patients were included in the abnormal ABI group.

In the present study, we measured ABI and FMD only once. It is well known that FMD shows circadian variation, especially postprandial changes, whereas ABI is almost constant. We measured FMD at a fixed time. In the present study, FMD was examined in 132 of the 389 subjects on a separate day. We confirmed that FMD assessed on that day was also significantly smaller in the borderline group than in the normal group (3.2±2.6% vs. 5.1±2.7%, P=0.04) (Figure S1). However, we cannot deny the possibility that day-to-day variation and differences in the time of measuring FMD affected the relationship between FMD and ABI.

In conclusion, a borderline ABI value of 0.91–0.99 is associated with endothelial dysfunction, even when vascular smooth muscle function and brachial IMT are maintained in the normal ranges. Measurement of ABI is a simple and cost-effective method for obtaining additional information on the grade of atherosclerosis beyond the assessment of PAD. Further large-scale trials and long-term studies are needed to confirm the relationship between changes in ABI and FMD and to evaluate the effects of interventions for decreasing or maintaining a patient’s ABI on endothelial function and cardiovascular events.

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**Financial Disclosures**

None.

**References**

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Supplementary Files

Supplementary File 1.

Figure S1. Results of flow-mediated vasodilation examined in 132 of the 389 subjects on a separate day. Please find supplementary file(s): http://dx.doi.org/10.1253/circj.CJ-14-0165