The Clinical Applicability of Albuminuria Testing in Japanese Hypertensive Patients: The AVA-E Study

Yoshihiro Tani, Masaaki Nakayama, Makoto Kanno, Hiroshi Kimura, Kimio Watanabe, Kenichi Tanaka, Yoshimitsu Hayashi, Koichi Asahi, Kunitoshi Iseki and Tsuyoshi Watanabe

Abstract

Objective Albuminuria is thought to reflect generalized endothelial dysfunction. In hypertensive patients, albuminuria increases the risk of cardiovascular disease (CVD) events. Therefore, screening for albuminuria is critical for stratifying risks in hypertensive patients. However, a limited number of Japanese studies have performed quantitative examinations of albuminuria. The objective of this study was to examine the utility of the CLINITEK MICROALB CREATININE TEST for albuminuria screening.

Materials The CLINITEK MICROALB CREATININE TEST consists of a urine test strip that assesses albumin excretion corrected for the urine creatinine levels in only 60 seconds without the need for any special facilities. The CLINITEK MICROALB CREATININE TEST was performed in 5,647 Japanese hypertensive patients, excluding diabetic patients, and the clinical significance of the test was evaluated.

Results According to the CLINITEK MICROALB CREATININE TEST, the A1 (albumin creatinine ratio: ACR <30 mg/g·creatinine), A2 (ACR 30-299 mg/g·creatinine) and A3 (ACR ≥300 mg/g·creatinine) levels of albuminuria were present in 61.2%, 32.5% and 6.3% of the patients surveyed, respectively. The proportions of A2 and A3 patients increased with chronic kidney disease (CKD) stage, blood pressure, age and previous history of CVD. According to a multivariate logistic regression analysis, the A2 and A3 levels of albuminuria were found to be independently associated with a previous history of CVD (odds ratio: 1.36, 95% confidence interval: 1.08-1.72, p<0.01) after adjusting for age, diabetes, blood pressure and estimated glomerular filtration rate (eGFR).

Conclusion In hypertensive patients, the A2 and A3 levels of albuminuria on the CLINITEK MICROALB CREATININE TEST are associated with a previous history of CVD, independent of eGFR. Therefore, by reflecting the status of systemic vascular injury, this test may help to perform CVD risk stratification.

Key words: albuminuria, hypertension, screening test, risk factors, CLINITEK MICROALB CREATININE TEST

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Introduction

Hypertension is a leading risk factor for cardiovascular disease (CVD) and all-cause mortality (1-3). Moreover, the risk for these events among hypertensive patients is further increased by the presence of albuminuria (4, 5). Albuminuria reflects vascular damage and appears to be a marker of early arterial disease (6). Furthermore, albuminuria is hypothesized to reflect morphological and functional derangements of strained vessels in the kidneys, coronary arteries and perforating vessels in the brain (7). Since albuminuria is an independent risk factor for CVD and chronic kidney disease (CKD) morbidity and mortality (8-17), then assessing

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the presence of albuminuria is important for stratifying risks in hypertensive patients.

A limited number of facilities in Japan currently perform quantitative examinations to detect albuminuria. Therefore, not all Japanese hypertensive patients have the opportunity to have their urine checked.

The CLINITEK MICROALB CREATININE TEST is a test strip that examines urine albumin excretion corrected for the creatinine levels. The entire examination takes 60 seconds and does not require special facilities. In this study, the albuminuria levels were examined in hypertensive patients using the CLINITEK MICROALB CREATININE TEST in order to investigate the test’s clinical utility in such patients.

Materials and Methods

Study population

A total of 8,628 Japanese hypertensive outpatients who underwent testing using the CLINITEK MICROALB CREATININE TEST between September 2009 and March 2010 were registered in the Albuminuria Validation Analysis-Epidemiological (AVA-E) Study (planned and conducted by Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan). The data were collected via the internet from 639 cooperating physicians nationwide. The present study is a survey of 5,647 of the total 8,628 hypertensive patients, excluding those with diabetes. The study protocol complied with the Helsinki Declaration and was approved by the ethics committee of Fukushima Medical University School of Medicine. All patients gave their informed consent to participate in this study.

Blood pressure measurement

Blood pressure was measured using a standard mercury sphygmomanometer with the subject in the sitting position. Three measurements were obtained, separated by at least 5-minute intervals, and the mean of the three measurements was used for the analysis.

CLINITEK MICROALB CREATININE TEST

Similar to conventional dipstick test strips for urinalyses, the CLINITEK MICROALB CREATININE TEST consists of a test paper attached to a plastic strip designed to detect albumin and creatinine. The principle of detecting albuminuria is based on the protein error observed using a high-affinity dye-binding method (18). Creatinine detection relies on the peroxidase-like activity of the copper-creatinine complex (19). A survey of albuminuria in 5,647 outpatients with essential hypertension was conducted. The strips were dipped in well-stirred, fresh urine specimens, and the excess urine was removed. Albumin and creatinine were assayed using colorimetry for 50 and 60 seconds, respectively. The colorimetric assays were performed under a bright light source of 1,000±100 lux. A visual estimate of the albumin concentrations was performed using a color comparison chart with nominal albumin values of 10, 30, 80 or 150 mg/L. Similarly, a visual estimate of the creatinine concentrations was performed using a color comparison chart with nominal creatinine values of 10, 50, 100, 200 or 300 mg/L. The results were categorized as A1 (ACR <30 mg/g·creatinine), A2 (ACR 30-299 mg/g·creatinine) or A3 (ACR ≥300 mg/g·creatinine).

Data collection

For each patient, non-fasting blood samples were collected from the antecubital vein at the outpatient clinic to determine the serum creatinine, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG) and plasma glucose levels. The estimated glomerular filtration rate (eGFR) formula proposed by the Japanese Society of Nephrology was used to calculate the eGFR as follows: 194× serum creatinine−1.094× age−0.287 (×0.739 if female) (20). The serum creatinine levels were measured using an enzyme-based method.

Previous myocardial infarctions (MIs) were diagnosed based on changes in electrocardiogram and laboratory data suggesting infarction with accompanying clinical symptoms or documentation on coronary angiography. Previous strokes were confirmed using computed tomography (CT) or magnetic resonance imaging (MRI) with or without the presence of clinical neurological disorders. Hyperlipidemia was defined as the use of lipid-lowering drugs or any of the following laboratory data: LDL-C >140 mg/dL, TG >150 mg/dL or HDL-C <40 and <50 mg/dL in men and women, respectively.

Statistical analysis

All variables are expressed as the mean ± standard deviation (SD). Pearson’s rank correlation test was used to estimate relationships between variables. A multinomial logistic regression analysis was used to estimate the odds ratio (OR) and 95% confidence intervals (CI). The statistical analyses were performed using the SPSS Statistics version 17.0 software program (SPSS Japan, Tokyo, Japan).

Results

Table 1 shows the clinical characteristics of the 5,647 hypertensive patients assessed in this study. According to the CLINITEK MICROALB CREATININE TEST, the albuminuria levels were in the A1 category in 61.2% of the subjects, the A2 category in 32.5% of the subjects and the A3 category in 6.3% of the subjects.

The physicians who participated in this survey included general internists (62.4%), cardiologists (16.4%), nephrologists (3.4%) and others (17.8%). The subjects were assessed in clinics (89.6%), general hospitals (9.5%), national or public hospitals (0.6%) and university hospitals (0.3%). Subjects from various parts of Japan were assessed, including those from the north (Hokkaido, 3.2%; Tohoku, 11.8%), central (Kanto, 30.0%; Chubu, 16.1%), west (Kinki, 20.2%; Chu-
Table 1. Clinical Characteristics of Hypertensive Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hypertensive patients, without diabetes (n=5,647)</th>
<th>All (n=8,628)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, n (%)</td>
<td>2,688 (47.6%)</td>
<td>4,383 (50.8%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.8±11.6</td>
<td>67.4±11.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1±3.5</td>
<td>24.5±3.7</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>908 (16.1%)</td>
<td>1,525 (17.7%)</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>2,968 (52.6%)</td>
<td>4,924 (57.1%)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>0 (-%)</td>
<td>2,981 (34.6%)</td>
</tr>
<tr>
<td>CVD, n (%)</td>
<td>381 (6.7%)</td>
<td>731 (8.5%)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>138.0±15.8</td>
<td>138.0±15.7</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79.0±18.6</td>
<td>78.6±11.3</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>69.5±18.6</td>
<td>69.8±19.9</td>
</tr>
</tbody>
</table>

CLINITEK MICROALB CREATININE TEST, n (%)

- A1 (ACR <30 mg/g creatinine) 3,454 (61.2%) 4,922 (57.0%)
- A2 (ACR 30-299 mg/g creatinine) 1,835 (32.5%) 3,033 (35.2%)
- A3 (ACR ≥300 mg/g creatinine) 358 (6.3%) 673 (7.8%)

Numeric data are presented as means ± standard deviation.
Categorical variables are expressed as percentages.
BMI: body mass index, CVD: cardiovascular disease, eGFR: estimated glomerular filtration rate

Figure 1. Results of the CLINITEK MICROALB CREATININE TEST stratified by CKD stage. The proportions of patients with A2 (ACR 30-299 mg/g creatinine) and A3 (ACR≥300 mg/g creatinine) levels of albuminuria increased with advancing CKD stage. CKD stage 1, eGFR≥90 mL/min/1.73 m²; Stage 2, eGFR 60-89 mL/min/1.73 m²; Stage 3, eGFR 30-59; Stage 4, eGFR 15-29 mL/min/1.73 m²; Stage 5, eGFR < 15 mL/min/1.73 m². CKD: chronic kidney disease, eGFR: estimated glomerular filtration rate

Figure 2. Results of the CLINITEK MICROALB CREATININE TEST stratified by systolic blood pressure. The proportions of patients with A2 (ACR 30-299 mg/g creatinine) and A3 (ACR≥300 mg/g creatinine) levels of albuminuria increased with increasing systolic blood pressure. BP: blood pressure

Among those with previous histories of either MI or stroke and highest among those with previous histories of both MI and stroke (Fig. 4).

As presented in Table 2, the logistic regression analysis showed a number of independent predictors of the A2 and A3 levels of albuminuria, including age (OR 1.01, 95% CI 1.01-1.02, p<0.01), current smoking habits (OR 1.28, 95% CI 1.07-1.63, p<0.01), CVD (OR 1.14, 95% CI 1.12-1.77, p<0.01), systolic blood pressure (OR 1.01, 95% CI 1.00-1.01, p<0.01) and eGFR (OR 0.98, 95% CI 0.98-0.99, p<0.01).

A multivariate logistic regression analysis using a previous history of CVD as the dependent variable was also performed (Table 3). This analysis showed that the presence of...
patients. The proportions of patients with A2 (ACR 30-299 mg/g-creatinine) and A3 (ACR≥300 mg/g-creatinine) levels of albuminuria increased with increasing age. CVD: cardiovascular disease

**Figure 3.** Results of the CLINITEK MICROALB CREATININE TEST stratified by age quartiles. The proportions of patients with A2 (ACR 30-299 mg/g-creatinine) and A3 (ACR≥300 mg/g-creatinine) levels of albuminuria increased with increasing age. CVD: cardiovascular disease

**Figure 4.** Results of the CLINITEK MICROALB CREATININE TEST stratified by CVD category. The proportions of patients with A2 (ACR 30-299 mg/g-creatinine) and A3 (ACR≥300 mg/g-creatinine) levels of albuminuria were higher among those with a previous history of either MI or stroke and highest among those with a previous history of both MI and stroke. CVD: cardiovascular disease, MI: myocardial infarction

Table 2. Adjusted Odds Ratio for A2 (ACR 30-299 mg/g-creatinine) / A3 (ACR≥300 mg/g-creatinine) according to the CLINITEK MICROALB CREATININE TEST on Logistic Regression Analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male)</td>
<td>0.99</td>
<td>0.87-1.13</td>
<td>0.99</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.01</td>
<td>1.01-1.02</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>1.00</td>
<td>0.98-1.02</td>
<td>0.69</td>
</tr>
<tr>
<td>Current smoking</td>
<td>1.28</td>
<td>1.07-1.53</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>1.05</td>
<td>0.92-1.19</td>
<td>0.42</td>
</tr>
<tr>
<td>CVD</td>
<td>1.14</td>
<td>1.12-1.77</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>1.01</td>
<td>1.00-1.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>0.99</td>
<td>0.99-1.00</td>
<td>0.54</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>0.98</td>
<td>0.98-0.99</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

BMI: body mass index, CVD: cardiovascular disease, eGFR: estimated glomerular filtration rate, OR: odds ratio, CI: confidence interval

A2 or A3 levels of albuminuria, as assessed with the CLINITEK MICROALB CREATININE TEST, is associated with a greater likelihood of having a previous history of CVD (OR 1.36, 95% CI 1.08-1.72, p<0.01) after adjusting for sex, age, BMI, current smoking habits, hyperlipidemia, systolic blood pressure, diastolic blood pressure and eGFR.

**Discussion**

To the best of our knowledge, this study is the first to show that A2 and A3 levels of albuminuria, as assessed with the CLINITEK MICROALB CREATININE TEST, are associated with a previous history of CVD, independent of eGFR. The results indicate that the CLINITEK MICROALB CREATININE TEST may be equivalent to albuminuria measurement in its utility for stratifying risks in hypertensive patients.

Vascular endothelial damage caused by hypertension results in albuminuria (6), and increased intraglomerular pressure due to hypertension and a low eGFR is hypothesized to result in albuminuria (7). Accordingly, in this study, the prevalence of A2 and A3 levels of albuminuria assessed on the CLINITEK MICROALB CREATININE TEST increased in association with declines in the kidney function and increases in blood pressure. Moreover, A2 and A3 levels of albuminuria on the CLINITEK MICROALB CREATININE TEST were found to be correlated with the CKD stage, blood pressure category and age category. Although albuminuria was evaluated using the CLINITEK MICROALB CREATININE TEST, the results were similar to those of a previous study of albuminuria (21). Moreover, A2 and A3 levels of albuminuria, as assessed with the CLINITEK MICROALB CREATININE TEST, were found to be associated with a previous history of CVD, independent of age, blood pressure and eGFR. These findings suggest that concurrent assessment of the albuminuria levels using the CLINITEK MICROALB CREATININE TEST may be important for accurately predicting the risk of CVD.

Although there is evidence of an increased risk of CVD due to albuminuria, nondiabetic patients in Japan are not typically screened for albuminuria because the value of screening in these patients is unclear. Additionally, therapy in this setting has not been shown to be useful or cost-effective (22). Since the CLINITEK MICROALB CREATININE TEST is easy, quick and inexpensive compared with quantitative albuminuria measurement, it may be used as a substitute for quantitative albuminuria measurement for purposes of CVD risk stratification.

The unique point of the present survey is that as many as 89.6% of the physicians who participated in this survey were general practitioners and the patients were outpatients...
with a diagnosis of essential hypertension. According to the CLINITEK MICROALBUMIN CREATININE TEST, the albuminuria levels were in the A1 category in 61.2%, the A2 category in 32.5% and the A3 category in 6.3% of the 5,647 hypertensive subjects, excluding those with diabetes. Moreover, the levels were in the A1 category in 57.0%, the A2 category in 35.2% and the A3 category in 7.8% of the total 8,628 hypertensive subjects, including those with diabetes. However, it should be noted that the A2 and A3 levels of albuminuria, as detected on the CLINITEK MICROALB CREATININE TEST, do not always indicate positive albuminuria. Indeed, previous studies have reported that the positive predictive value of the CLINITEK MICROALB CREATININE TEST is 53% in the general population (23) and 46% among diabetic patients (24). Quantitative examinations of albuminuria should be performed among patients with A2 and A3 levels of albuminuria according to the CLINITEK MICROALB CREATININE TEST to assess the level of agreement between the two measurements.

The present study is associated with several limitations. First, since the study design was cross-sectional, further investigation is needed to clarify the association between the CLINITEK MICROALB CREATININE TEST results and the risk of CVD. The cost-benefit balance of assessing the albuminuria levels using the CLINITEK MICROALBUMIN CREATININE TEST to predict CVD morbidity and mortality warrants further study. Second, urine samples were collected on a single occasion, and the collection times differed among the patients. Third, the prevalence of albuminuria is higher in men; however, it did not differ between the sexes in the present study. This lack of difference may be due to the older mean age of the present sample (64.8±11.9 years in the men vs. 69.5±10.9 years in the women, p<0.01) and the low rate of RAS-I (renin-angiotensin system inhibitor) therapy use (66.9% in the men vs. 59.9% in the women, p<0.01). However, the present study was cross-sectional and precise antihypertensive prescription records were not obtained; therefore, further investigation is needed to clarify the effects of antihypertensive agents on the prevalence of albuminuria. Fourth, Iseki et al. reported that cardiac disease and strokes are found in 6.0% and 3.3% of the general population, respectively (25). It appears that there was a low prevalence of a previous history of CVD (cardiac disease in 2.3% of the patients and stroke in 4.8% of the patients) in this study compared with the findings of that report. It is difficult to compare the prevalence of CVD because the patients’ characteristics differed. One reason for the difference may be that patients with diabetes were excluded from the present study. Finally, hypertension is a leading risk factor for CVD (1-3). Systolic blood pressure was found to be associated with a previous history of CVD, as shown in Table 3; however, the odds ratio was less than 1. Since the subjects had been treated with antihypertensives, the current systolic blood pressure values are not necessarily associated with CVD.

In conclusion, the A2 and A3 levels of albuminuria as assessed according to the CLINITEK MICROALB CREATININE TEST are associated with a previous history of CVD, independent of eGFR. This finding suggests that the CLINITEK MICROALB CREATININE TEST might reflect systemic vascular injury and may be useful in the stratification of CVD risk.

The authors state that they have no Conflict of Interest (COI).


