The Characteristics of New Semi-Quantitative Method for Diagnosing Proteinuria by Using Random Urine Samples

Cheng-Jui Lin,^{1,2} Han-Hsiang Chen,^{1,2} Chi-Feng Pan,^{1,2} Wen-Shyang Hsieh,³
Hsin-Tsung Ho,^{2,3} and Chih-Jen Wu^{1,2,4*}

¹*Division of Nephrology, Department of Internal Medicine, Mackay Memorial Hospital, Taipei, Taiwan*

²*Mackay Medicine, Nursing and Management College, Taipei, Taiwan*

³*Department of Laboratory Medicine, Mackay Memorial Hospital, Taipei, Taiwan*

⁴*Graduate Institute of Medical Science, Taipei Medical University, Taipei, Taiwan*

We assessed the characteristics of the new semi-quantitative test paper (Clinitek ATLAS Pro¹²) using random urine samples. Three hundred urine samples were analyzed using either the new test paper, conventional dipsticks, quantitative (P/C ratio), or immunological quantitative methods (A/C ratio). Our study showed that the new test paper is highly sensitive and specific for the detection of urinary protein. The new test paper also detected the urine protein more accurately than the conven-

tional test and has a lower false-positive rate. In addition, the new test paper detected 14 of the 300 patients (4.7%) as dilute urine samples needing reassessment. Seventeen of the 300 samples tested were negative with conventional dipsticks but positive with the new test paper. The new semi-quantitative test paper not only has higher sensitivity than the conventional dipstick method, but also has potential to detect dilute samples. *J. Clin. Lab. Anal.* 25:14–19, 2011. © 2011 Wiley-Liss, Inc.

Key words: chronic kidney disease; microalbuminuria; proteinuria

INTRODUCTION

The incidence and prevalence of end-stage renal disease (ESRD) are increasing (1), and it has become an important social and economic burden worldwide. To reduce the incidence of ESRD, medical intervention that enables early prevention or a delay in progression of chronic kidney disease (CKD) toward ESRD is extremely important. Microalbuminuria can be an early sign of kidney disease or kidney damage. Growing evidence indicate that microalbuminuria is a useful prognostic marker for cardiovascular risk in diabetic and nondiabetic subjects (2–5). Screening of urine protein is recommended for high-risk groups, including the elderly and persons with hypertension, family history of diabetes, or CKD (6–9). Thus, early detection of urine protein is a key issue.

The conventional dipstick urinalysis is a rapid and convenient tool for the diagnosis of proteinuria but has some limitations because it provides only qualitative results and is unable to detect urine microalbumin. The quantification method (which assesses the protein to creatinine ratio (P/C ratio) or the albumin to creatinine

ratio (A/C ratio)) is more reliable due to the reduction in the biological variation (10). It is also the most accurate method to diagnose proteinuria and microalbuminuria, and have replaced quantitative measurements of 24-hr excretion of urine protein to diagnose renal disease.

Clinitek ATLAS Pro¹² (Siemens Medical Solutions, Tarrytown, NY), a new semi-quantitative urine test paper, incorporates dual protein reagent pads—“Protein Low” and “Protein high”. The protein-low test can accurately and specifically determine albumin. The new test paper is unique in that it includes a test pad for creatinine allowing for the semi-quantitative estimation of P/C ratio (11,12).

Cheng-Jui Lin and Han-Hsiang Chen contributed equally to this article.

*Correspondence to: Chih-Jen Wu, Division of Nephrology, Department of Internal Medicine, Mackay Memorial Hospital, 92 Chung San North Road, Section 2, Taipei 104, Taiwan.
E-mail: wcjyali@yahoo.com.tw

Received 14 November 2009; Accepted 17 February 2010

DOI 10.1002/jcla.20376

Published online in Wiley Online Library (wileyonlinelibrary.com).

Our study objective was to evaluate the characteristics of the new test paper by using random urine samples.

MATERIALS AND METHODS

Subjects

Three hundred stable outpatients from a single medical center were enrolled in this study between January and April 2008. These patients had a medical history of type 2 diabetes, hypertension, cardiovascular disease, or CKD. Randomly collected urine samples were tested using the new test paper (Clinitek ATLAS Pro¹² method), the conventional dipsticks, the quantitative method (P/C ratio), and the immunological quantitative method (A/C ratio). We compared protein values measured qualitatively using the new test paper and the conventional dipstick with qualitative and quantitative values of protein, creatinine, and the P/C ratio measured using the new test paper. Urine samples were classified as dilute if creatinine level was 10 mg/dl and the protein concentration was normal.

Method of Urinalysis

The Clinitek ATLAS PRO¹² Reagent Pak system (Siemens Medical Solutions) includes a new protein-low reagent that allows "low level" detection of urine albumin at 15 mg/dl in addition to the current protein reagent used for the detection of "high-level" protein at 30 mg/dl. This system uses a new creatinine reagent for the estimation of the P/C ratio of the urine specimen. The use of the P/C ratio can assist in the diagnosis of kidney function by minimizing the impact of changes in the protein result attributable to exercise, orthostatic proteinuria, diuresis, and urine concentration. The ratio allows for the use of single-void specimens in the discrimination of normal and abnormal levels of protein. P/C ratios of >150 mg/g are considered abnormal.

Quality control of the new strip: Routinely, test is carried out at least at two levels (negative/low and positive) of an appropriate commercially available control to confirm the performance of the Reagent Pak. Controls may also be randomly hidden in each batch of specimens tested. Water should not be used as a negative control. Each laboratory should establish its own goals for acceptable standards of performance, and should question handling and testing procedures if these standards are not met.

The assay for low-level protein (albumin) is based on dye binding using the high-affinity dye *Bis* (3', 3'' diiodo-4', 4''-dihydroxy-5', 5''-dinitrophenyl)-3,4,5,6-tetrabromosulfonaphthalein). At a constant pH, a blue color develops due to the presence of albumin; the intensity of the color is proportional to the albumin concentration. The assay for

creatinine is based on the peroxidase-like activity of copper creatinine complexes that catalyze the reaction of diisopropylbenzene dihydroperoxide and 3, 3', 5, 5'-tetramethyl-benzidine.

Table 1 shows the quantitative values of protein, creatinine, and P/C ratio corresponding to each color block on the new test paper. For conventional dipstick, the lowest limitation to detect urine protein is 15 mg/dl. The cut-off value of conventional dipstick is set at 15 mg/dl. The lowest value to detect urinary protein is 8 mg/dl for the new test paper and it is choose as cut-off level while comparing to quantitative method. In addition, the cut-off value of protein semi-quantification of new test paper is 30, 100, 300, and 1,000. To compare with the quantitative method under the same basis, the range used reflects the mid-point between report result levels. The calculation formula is $65 = 30 + (100 - 30) / 2$. Therefore, the relative cut-off value in quantitative method is 30–64.9, 65–199.9, 200–399.9, and 400 mg/dl. For P/C ratio, the normal P/C ratio is <80 mg/g in quantitative method. The cut-off value of the new test on P/C ratio is set at 150, 300, and >500 mg/g originally. Thus, to compare the new test with the quantitative method under the same basis, the cut-off value of quantitative method was set as 80–299.9, 300–499.9, and >500 mg/g.

The following measurements were performed for each urinary specimen: conventional dipstick by the method of Medi-Test Uryxxon-Stick10 (Combi-10SGL, Dueren, Germany); urine protein quantification (P/C) by using SYNCHRON LX systems (Beckman Coulter Inc., California), which assessed micro total protein by

TABLE 1. Quantitative Values Correspond to the Color Blocks for Protein, Creatinine and P/C Ratio in New Test Paper

	New test paper (mg/dl)	Quantitative value (mg/dl)
Albumin	8	8.0
	15	8–29.9
Protein	30	30–64.9
	100	65–199.9
	300	200–399.9
	1,000	400.0
Creatinine	10	25.0
	50	25–74.9
	100	75–149.9
	200	150–249.9
	300	250.0
	Results of P/C ratio (mg/g)	Quantitative value (mg/dl)
P/C ratio	–	Protein normal
	Dilution	Creatinine <25 mg/dl
	Normal	<80
	150	80–299.9
	300	300–499.9
	≥500	≥500

TABLE 2. Baseline Patient Demographics

Patient demographics (n = 300)	
Median age (years)	56 ± 12.7
Men	116 (38.7%)
Women	184 (61.3%)
Hypertension	97 (32.3%)
Diabetes	94 (31.3%)
Cardiovascular disease	5 (1.67%)
Chronic kidney disease	96 (32%)
Urea nitrogen (mg/dl)	24.4 ± 14.4
Creatinine (mg/dl)	1.93 ± 1.06
eGFR (ml/min)	61.7 ± 29.0
Systolic pressure (mmHg)	128.7 ± 11.7
Diastolic pressure (mmHg)	75.2 ± 8.0

Values within parenthesis represent percentages.

M-TP reagent (13). Protein in the sample reacts with pyrogallol red and molybdate to form a purple color complex that has a maximum absorbance at 600 nm. Urine creatinine was determined by means of Jaffe rate method (14). Creatinine in the sample combines with the reagent to produce a red color complex that has a maximum absorbance at 520 nm; urine microalbumin quantification (A/C) using the immunological quantitative method (IMAGE MA test; Beckman Coulter Inc.).

In this study, the estimated GFR value was calculated by Modification of Diet in Renal Disease study equation (15). When creatinine is in mg/dl (conventional units), this equation is: estimated glomerular filtration rate (eGFR; ml/min/1.73 m²) = $175 \times \text{Scr}^{-1.154} \times \text{age}^{-0.203} \times 0.742$ (if women). The calculation of sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV), positive likelihood ratios (LR(+)), and negative likelihood ratios (LR(-)) were performed to assess the diagnostic accuracy for the absence and presence of proteinuria.

RESULTS

Patient demographics are shown in Table 2. Of the 300 study participants (mean age: 56 ± 12.7 years; 116 males and 184 females), 97 (32.3%) had hypertension, 94 (31.3%) had diabetes, 5 (1.67%) had cardiovascular disease, and 96 (32%) had CKD. Mean serum urea nitrogen and creatinine were 24.4 ± 14.4 and 1.93 ± 1.06 mg/dl, respectively. Mean systolic blood pressure and diastolic pressure were 128.7 ± 11.7 and 75.2 ± 8.0 mmHg, respectively. Mean eGFR for these patients was 61.7 ± 29.0 ml/min.

Correlation Between the Conventional Test and Quantitative Method

Table 3 shows the results for protein assessment obtained with the conventional dipstick and quantitative

TABLE 3. Correlation Between Protein Test Portion of Conventional Dipstick and Quantitative Method

	Urinary total protein conc. (mg/dl)					
	<15	15–245	25–64.9	65–1995	≥200	Total
Conventional test paper, protein test portion (mg/dl)						
–	140	21	1			162
±		16	6			22
1+(30)			33	1		34
2+(100)		1	12	41		54
3+(≥300)			1	3	24	28
Total	140	38	53	45	24	300
	Quantitative method (mg/dl) ^a					
	Negative (<15)		Positive (≥15)			Total
Conventional test paper, protein test portion (mg/dl)						
Negative (–)	140		22			162
Positive (≥ ±)	0		138			138
Total	140		160			300

^aSensitivity = 86.3% (95% CI: 80.5–92.0); specificity = 100% (95% CI: 100–100) positive predictive value = 100% (95% CI: 100–100); negative predictive value = 86.4% (95% CI: 80.5–92.3); LR(+) = –; LR(–) = 0.14 (95% CI: 0.00–0.57); rate of complete coincidence = 84.7%; rate of coincidence = 92.67%.

method. Proteinuria greater than 15 mg/dl was regarded as a positive result in the quantitative method. The sensitivity and specificity of the conventional dipstick for detecting proteinuria were 86.3% (95% CI: 80.5–92.0) and 100% (95% CI: 100–100), respectively, relative to the quantitative method. The PPV was 100% (95% CI: 100–100), NPV 86.4% (95% CI: 80.5–92.3), LR(+) - and LR(–) 0.14 (95% CI: 0.00–0.57).

Correlation Between the New Test Paper and Quantitative Method

Protein

Table 4 compares the protein measurements obtained with the quantitative method and the new test paper. In this case, a protein level of 8 mg/dl assessed using the quantitative method was regarded as a positive result. The sensitivity and specificity of the new test paper for detecting proteinuria were 93.3% (95% CI: 89.1–97.5) and 91.6% (95% CI: 87.4–95.8), respectively, relative to the quantitative method. The PPV was 89.9% (95% CI: 84.9–94.9), NPV = 94.4% (95% CI: 90.8–98.0), LR(+) = 11.06 (95% CI: 7.43–14.69), and LR(–) = 0.07 (95% CI: 0.00–0.38).

P/C ratio

Table 5 compares the P/C ratio values between the new test paper and the quantitative method. A positive result was regarded as greater than 80 mg/g Cr. The

TABLE 4. Correlation Between Protein Test Portion of New Test Paper and Quantitative Method

	Quantitative method (mg/dl)					Total
	Protein (low)		Protein (high)			
	<8	8–29.9	65–199.9	≥200		
New test paper, protein test portion (mg/dl)						
–	152	9				161
Low/15	14	17				31
30		2	25	3		30
100		1	9	42	1	53
≥300				2	23	25
Total	166	29	34	47	24	300
	Quantitative method (mg/dl) ^a					Total
	Negative (<8)		Positive (≥8)			
New test paper, protein test, portion (mg/dl)						
Negative(–)		152		9		161
Positive (≥ ±)		14		125		139
Total		166		134		300

^aSensitivity = 93.3% (95% CI: 89.1–97.5); specificity = 91.6% (95% CI: 87.4–95.8); positive predictive value = 89.9% (95% CI: 84.9–94.9); negative predictive value = 94.4% (95% CI: 90.8–98.0); LR(+) = 11.06 (95% CI: 7.43–14.69); LR(–) = 0.07 (95% CI: 0.00–0.38); rate of coincidence = 92.3%; and rate of complete coincidence = 86.3%.

TABLE 5. Correlation of P/C Ratio Between New Test Paper and Quantitative Method

PJO(quantification) (mg/g)(80 mg/gCr was the basis of negative and positive result) ^a						
	Normal dilution	<80	80–299.9	300–499.9	≥500	Total
New test paper, P/C ratio (mg/g)						
Normal dilution	14					14
Normal		127	2			129
150		21	15	2	1	39
300			7	9	19	35
>500			2	1	80	83
Total	14	148	26	12	100	300
Quantitative method (mg/g)						
		Negative (<80)		Positive (≥80)		Total
New test paper P/Cr (mg/g)						
Negative (Normal)		127		2		129
Positive (≥150)		21		136		157
Total		148		138		286

^aWhen qualitative data with P/C ratio of 150 mg/g or higher is taken as positive; sensitivity = 98.6% (95% CI: 96.6–100.0); specificity = 85.8% (95% CI: 80.2–91.4); positive predictive value = 86.6% (95% CI: 81.3–91.9); negative predictive value = 98.4% (95% CI: 96.3–100); LR(+) = 6.95 (95% CI: 4.00–9.89); LR(–) = 0.02 (95% CI: 0.00–0.17); and the rate of complete coincidence = 98.6%. The false-positive and false-negative rates were 7.3% and 0.7%, respectively.

TABLE 6. Seventeen of the 300 Samples, Diagnosed as Negative by Using Conventional Dipstick but Positive by Immunological Quantitative Methods (A/C ratio ≥80 mg/g), Were Re-Assessed by the New Test Paper

Num	New test paper P/C (mg/g)	Immunological method A/C (mg/g)	Conventional dipstick (mg/dl)
1	150	86.9	–
2	150	95.3	–
3	150	113.9	–
4	300	114.0	–
5	Normal	120.1	–
6	150	122.5	–
7	Normal	124.9	–
8	150	132.2	–
9	300	139.9	–
10	150	155.6	–
11	300	201.8	–
12	Dilute	206.8	–
13	≥500	325.9	–
14	150	332.2	–
15	300	340.9	–
16	300	401.8	–
17	150	501.7	–

sensitivity and specificity on calculating the P/C ratio using the new test paper were 98.6% (95% CI: 96.6–100.0) and 85.5% (95% CI: 80.2–91.4), respectively, relative to the quantitative method. The PPV was 86.6% (95% CI: 81.3–91.9), NPV = 98.4% (95% CI: 96.3–100), LR(+) = 6.95 (95% CI: 4.00–9.89, and LR(–) = 0.02 (95% CI: 0.00–0.17).

False-negative and dilute samples

Seventeen of the 300 urine samples, diagnosed as negative by conventional dipstick but positive by immunological quantitative methods (A/C ratio ≥80 mg/g), were assessed by the new test paper (Table 6). Of these 17 samples, 15 samples were diagnosed as abnormal (including 1 dilute sample) and only 2 yielded negative readings by the new semi-quantitative paper. In addition, urine samples obtained from 14 of the 300 patients (4.7%) with 10-mg/dl creatinine but normal protein concentration, as determined with the new test paper, were considered dilute. In these patients, the use of conventional dipsticks led to overestimation of protein levels; therefore, it was necessary to obtain fresh samples.

DISCUSSION

Our study demonstrated that the new semi-quantitative test (Clinitek ATLAS PRO¹²) is highly sensitive and specific for the detection of urinary protein. Furthermore, it has a lower false-positive rate than the conventional dipstick method.

CKD has become an important public health problem and is associated with increased mortality worldwide attributable to diverse causes (16,17). Proteinuria is not only a simple marker of kidney disease but also a major finding of CKD progression. It also has been reported to be associated with cardiovascular disease and death (18). Thus, reduction in proteinuria has been linked to improvement in both cardiovascular and renal outcomes (19). The population of CKD is growing gradually, and low awareness of CKD is an important factor in explaining this trends (20). Thus, early recognition of this disorder is crucial to reduce its impact on mortality rates (21).

Currently, traditional urinalysis performed by using dipstick has a sensitivity of detection for urine protein of approximately 15–30 mg/dl. Thus, the urine samples are easily influenced by urine specific gravity. In the clinic, it is therefore difficult for physicians to differentiate between concentrated samples and samples indicating pathological proteinuria due to kidney damage, if the diagnostic test reveals a protein trace. Consequently, a shortcoming of traditional urinalysis involving dipsticks that limits their use as a first-line screening test is the high incidence of false-negative results. Spot urine P/C ratio was used to overcome variation in random urine sample measurements and provided a more objective result (22).

In this study, we evaluated the application of the new test paper in random urine samples. This system not only provides general biochemistry analysis but also enables semi-quantification of proteinuria (P/C). On the basis of our results, the new test paper showed good sensitivity and specificity. This new system was able to detect urine protein at much lower levels than conventional dipsticks; this was because of the inclusion of a new “protein-low” reagent that allows “low level” detection of urine albumin at 15 mg/dl. The new test paper contains the existing protein reagent used in current tests for the detection of “high-level” protein at 30 mg/dl. In addition, a new creatinine reagent is used for the estimation of the P/C ratio of the urine specimen. From our data, the rate of complete coincidence for the detection of urine creatinine was 69%, relative to the quantitative method. However, the percentage of agreement $\pm$ one level was up to 98.33%. Our data showed a good correlation between creatinine measurements obtained using the new test paper and those obtained with the quantitative method (data not shown).

The new test showed high sensitivity and specificity (98.6% and 85.5%, respectively) for calculating the P/C ratio. The major characteristic of the new test is that it substitutes the P/C ratio for qualitative analysis by conventional dipstick. The use of the P/C ratio can assist

in the diagnosis of kidney function by minimizing the impact of changes in the protein result attributable to exercise, orthostatic proteinuria, diuresis, and urine concentration. A further benefit of the new test is that it reveals when recollection of urine is necessary because of the collection of dilute samples. In our study, approximately 4.7% (14 of the 300) of samples were considered dilute. This finding reveals that approximately 5% of general urine specimens are dilute, leading to urine protein overestimation and false-positive results in current urine screening tests that use conventional dipsticks.

In addition, we found that 17 of the 300 urine samples were negative using conventional dipsticks but positive when assessed with the quantitative method. Only two of these 17 samples were negative when analyzed by the new test. These findings indicate that the new strip can detect urine protein more accurately than the conventional test and has a lower false-positive rate. However, the study has limitations including small patient number and lack of clinical outcome correlation. The effect of urine glucose, red blood cell, and white blood cell on the accuracy of protein detection was also not studied. Therefore, further investigation is needed to resolve these questions.

CONCLUSIONS

Our results indicate that the new semi-quantitative test paper, Clinitek ATLAS Pro¹², has higher sensitivity combined with a lower false-negative rate than the conventional dipstick method. The new test paper also can specifically detect dilute samples.

ACKNOWLEDGMENTS

We thank Bayer, now part of Siemens Medical Solutions, for kindly providing the Clinitek ATLAS Pro¹² reagent strips. Although Bayer provided reagent strips for this study, they had no other input or involvement in this article.

There are no significant relationships with or financial interests in any companies related to this study or article.

We also thank all the patients involved in this study and Miss Fang-Ju Sun for providing statistical assistance.

REFERENCES

1. Locatelli F, Valderrabano F, Hoenich N, et al. Progress in dialysis technology: membrane selection and patient outcome. *Nephrol Dial Transplant* 2000;15:1133–1139.
2. Jensen JS, Feldt-Rasmussen B, Strandgaard S, et al. Arterial hypertension, microalbuminuria, and risk of ischemic heart disease. *Hypertension* 2000;35:898–903.

3. Gerstein HC, Mann JF, Yi Q, et al. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *J Am Med Assoc* 2001;286:421–426.
4. Hillege HL, Fidler V, Diercks GF, et al. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. *Circulation* 2002;106:1777–1782.
5. Culleton BF, Larson MG, Wilson PW, et al. Cardiovascular disease and mortality in a community-based cohort with mild renal insufficiency. *Kidney Int* 1999;56:2214–2219.
6. Boulware LE, Jaar BG, Tarver-Carr ME, et al. Screening for proteinuria in US adults—A cost-effectiveness analysis. *J Am Med Assoc* 2003;290:3101–3114.
7. Agrawal B, Berger A, Wolf K, et al. Microalbuminuria screening by reagent strip predicts cardiovascular risk in hypertension. *J Hypertens* 1996;14:223–228.
8. Scheid DC, McCarthy LH, Lawler FH, et al. Screening for microalbuminuria to prevent nephropathy in patients with diabetes. *J Fam Pract* 2001;50:661–668.
9. D Jong PE, Hillege HL, Pinto-Sietsma SJ, et al. Screening for microalbuminuria in the general population: a tool to detect subjects at risk for progressive renal failure in an early phase? *Nephrol Dial Transplant* 2003;18:10–13.
10. Price CP, Newall RG, Boyd JC. Use of protein:creatinine ratio measurements on random urine samples for prediction of significant proteinuria: a systemic review. *Clin Chem* 2005;51:1577–1586.
11. Croal BL, Finlay D, Davidson E, et al. Evaluation of the bayer multistix pro 10 LS point-of-care urine test. *Point Care* 2003;2:144–148.
12. Watanaba M, Funabiki K, Tsuge T, et al. Using protein/creatinine ratio in random urine. *J Lab Clin Anal* 2005;19:160–166.
13. Watanaba Y, Kamel S, Ohkuubo A, et al. Urine protein as measured with a pyrogallol red-molybdate complex, manually and in a hitachi 726 automated analyzer. *Clin Chem* 1986;328:1551.
14. Jaffe MZ. Über den Niederschlag weichen pikrinsäure in normalen Harn erzeugt und über eine neue Reaction des Kreatinins. *Z Physiol Chem* 1886;10:391.
15. Poggio ED, Wang X, Greene T, et al. Performance of the modification of diet in renal disease and Cockcroft-Gault equations in the estimation of GFR in health and in chronic kidney disease. *J Am Soc Nephrol* 2005;16:459–466.
16. Couser WG. Chronic kidney disease—the promise and the perils. *J Am Soc Nephrol* 2007;18:2803–2805.
17. Coresh J, Selvin E, Stevens LA, et al. Prevalence of chronic kidney disease in the United States. *JAMA* 2007;298:2038–2047.
18. Keane WF, Eknoyan G. Proteinuria, albuminuria, risk, assessment, detection, elimination (PARADE): a position paper of the National Kidney Foundation. *Am J Kidney Dis* 1999;33:1004–1010.
19. De Zeeuw D, Remuzzi G, Parving HH, et al. Proteinuria, a target for renoprotection in patients with type 2 diabetic nephropathy: lessons from RENAAL. *Kidney Int* 2004;65:2309–2320.
20. Hsu CC, Hwang SJ, Wen CP, et al. High prevalence and low awareness of CKD in Taiwan; a study on the relationship between serum creatinine and awareness from a nationally representative survey. *Am J Kidney Dis* 2006;48:727–738.
21. Stehouwer CD, Smulders YM. Microalbuminuria and risk for cardiovascular disease: Analysis of potential mechanisms. *J Am Soc Nephrol* 2006;17:2106–2111.
22. Schwab SJ, Christensen RL, Dougherty K, et al. Quantitation of proteinuria by the use of protein to creatinine ratios in single urine sample. *Arch Intern Med* 1987;147:943–944.