network assembly is encoded by the noncollagenous NC1 domains. *J Biol Chem* 2000; 275: 30716–30724
3. Yoshioka K, Hino S, Takemura T *et al*. Type IV Collagen a5 chain: normal distribution and abnormalities in X-linked Alport syndrome revealed by monoclonal antibody. *Am J Pathol* 1994; 144: 986–996
4. Cheong HI, Kashtan CE, Kim Y *et al*. Immunohistologic studies of type IV collagen in anterior lens capsules of patients with Alport syndrome. *Lab Invest* 1994; 70: 553–557
5. Peissel B, Geng L, Kalluri R *et al*. Comparative distribution of the a1 (IV), a5(IV) and a6(IV) collagen chains in normal human adult and fetal tissues and in kidneys from X-linked Alport syndrome patients. *J Clin Invest* 1995; 96: 1948–1957
6. Zehnder AF, Adams JC, Santi PA *et al*. Distribution of type IV collagen in the cochlea in Alport syndrome. *Arch Otolaryngol Head Neck Surg* 2005; 131: 1007–1013
7. Borza DB, Bondar O, Ninomiya Y *et al*. The NC1 domain of collagen IV encodes a novel network composed of the alpha 1, alpha 2, alpha 5, and alpha 6 chains in smooth muscle basement membranes. *J Biol Chem* 2001; 276: 28532–28540
8. Seki T, Naito I, Oohashi T *et al*. Differential expression of type IV collagen isoforms, a5(IV) and a6(IV) chains, in basement membranes surrounding smooth muscle cells. *Histochem Cell Biol* 1998; 110: 359–366
9. Gubler MC. Inherited diseases of the glomerular basement membrane. *Nat Clin Pract Nephrol* 2008; 4: 24–37
10. Gould DB, Phalan FC, van Mil SE *et al*. Role of COL4A1 in small-vessel disease and hemorrhagic stroke. *N Engl J Med* 2006; 354: 1489–1496
11. Gould DB, Phalan FC, Breedveld GJ *et al*. Mutations in Col4a1 cause perinatal cerebral hemorrhage and porencephaly. *Science* 2005; 308: 1167–1171
12. van der Knaap MS, Smit LM, Barkhof F *et al*. Neonatal porencephaly and adult stroke related to mutations in collagen IV A1. *Ann Neurol* 2006; 59: 504–511
13. Plaisier E, Gribouval O, Alamowitch S *et al*. COL4A1 mutations and hereditary angiopathy, nephropathy, aneurysms, and muscle cramps. *N Engl J Med* 2007; 357: 2687–2695
14. Vaicys C, Hunt CD, Heary RF. Ruptured intracranial aneurysm in an adolescent with Alport's syndrome—a new expression of type IV collagenopathy. *Surg Neurol* 2000; 54: 68–72
15. Lyons OTA, St. John ERC, Morales JP *et al*. Ruptured thoracoabdominal aortic aneurysm in a renal transplant patient with Alport's syndrome. *Ann Vasc Surg* 2007; 21: 816–818
16. Kashtan C, Butkowski R, Kleppel M *et al*. Posttransplant antiglomerular basement membrane nephritis in related Alport males with Alport syndrome. *J Lab Clin Med* 1990; 116: 508–515
17. Ding J, Zhou J, Tryggvason K *et al*. COL4A5 deletions in three patients with Alport syndrome and posttransplant antiglomerular basement membrane nephritis. *J Am Soc Nephrol* 1994; 5: 161–168
18. Tayel S, Kurczynski TW, Levine M *et al*. Marfanoid children: etiologic heterogeneity and cardiac findings. *Am J Dis Child* 1991; 145: 90–93
19. Kleppel MM, Fan W, Cheong H *et al*. Immunochemical studies of the Alport antigen. *Kidney Int* 1992; 41: 1629–1637
20. van der Loop FTL, Monnens LAH, Schroder CH *et al*. Identification of COL4A5 defects in Alport syndrome by immunochemistry of skin. *Kidney Int* 1999; 55: 1217–1224
21. Rheault MN, Kren SM, Thielen BK *et al*. Mouse model of X-linked Alport syndrome. *J Am Soc Nephrol* 2004; 15: 1466–1474
22. Januzzi JL, Isselbacher EM, Fattori R *et al*. Characterizing the young patient with aortic dissection: results from the International Registry of Aortic Dissection (IRAD). *J Am Coll Cardiol* 2004; 43: 665–669
23. Golledge J, Muller J, Daughtery A *et al*. Abdominal aortic aneurysm: pathogenesis and implications for management. *Arterioscler Thromb Vasc* 2006; 26: 2605–2613
24. Jais JP, Knebelmann B, Giatras I *et al*. X-linked Alport syndrome: natural history in 195 families and genotype-phenotype correlations in males. *J Am Soc Nephrol* 2000; 11: 649–657
25. Wolinksy H, Glagov S. A lamellar unit of aortic medial structure and function in mammals. *Circ Res* 1967; 20: 99–111
26. Dingemans KP, Teeling P, Lagendijk JH *et al*. Extracellular matrix of the human aortic media: an ultrastructural histochemical and immunohistochemical study of the adult aortic media. *Anat Rec* 2000; 258: 1–14
27. Sariola H, Viljanen T, Luosto R. Histological pattern and changes in extracellular matrix in aortic dissections. *J Clin Pathol* 1986; 39: 1074–1081
28. Pannu H, Avidan N, Tran-Fadulu V *et al*. Genetic basis of thoracic aortic aneurysms and dissections. *Ann N Y Acad Sci* 2006; 1085: 242–255
29. Judge DP, Dietz HC. Marfan's syndrome. *Lancet* 2005; 366: 1965–1976
30. Vogt BA, Birk PE, Panzarino V *et al*. Aortic dissection in young patients with chronic hypertension. *Am J Kidney Dis* 1999; 33: 374–378

Received for publication: 10.3.10; Accepted in revised form: 26.4.10

Nephrol Dial Transplant (2010) 25: 3560–3568
doi: 10.1093/ndt/gfq478
Advance Access publication 11 August 2010

Selection on albuminuria enhances the efficacy of screening for cardiovascular risk factors

Akin Özyilmaz[1], Stephan J. L. Bakker[1], Dick de Zeeuw[2], Paul E. de Jong[1], Ronald T. Gansevoort[1] and for the PREVEND Study Group

[1]Division of Nephrology, Department of Medicine and [2]Department of Clinical Pharmacology, University Medical Center Groningen and University of Groningen, Groningen, The Netherlands

Correspondence and offprint requests to: Akin Özyilmaz; E-mail: a.ozyilmaz@int.umcg.nl

Abstract

Background. As many subjects with a cardiovascular (CV) risk factor are undiagnosed, guidelines to prevent cardiovascular disease argue for case finding on those risk factors. Such an approach is, however, labour and cost intensive. An elevated urinary albumin loss is an early marker of vascular damage and is associated with an increased CV risk. As albuminuria is easy to measure, we tested whether a screening approach in which detailed risk factor measurement is done only after selection of subjects with an elevated albuminuria results in a higher yield of subjects at risk.

Methods. A random sample of the general population as investigated in the Prevention of Renal and Vascular End-Stage Disease study was used. Plasma glucose, blood pressure, serum cholesterol and renal function were measured in an overall random sample of the population, in subgroups according to their urinary albumin concentration (UAC) of one first morning urine void and in subgroups in whom the elevated albuminuria level was confirmed with two 24 h urine collections for measurement of urinary albumin excretion (UAE).

Results. In the overall population, the number of subjects with any newly found CV risk factor was higher than the number of subjects already known with any CV risk factor (n = 1331 versus 370; 39.2 versus 10.9%). The prevalence of subjects with any newly diagnosed CV risk factor was higher in the group of 267 subjects with a first morning UAC of ≥20 mg/L (61.0%; $P < 0.05$) compared to the overall population (39.2%). Although the sensitivity of a UAC ≥20 mg/L to detect a subject with at least one CV risk factor was relatively low (12%), the specificity was very high (96%). The positive predictive value was 70%. When the elevated UAC could be confirmed in two subsequent 24-h urine collections, the diagnostic yield still further improved.

Conclusion. The prevalence of undiagnosed CV risk factors in the general population is much higher than the prevalence of known risk factors. After a selection of subjects with an elevated albuminuria, the relative prevalence of subjects with newly diagnosed CV risk factors increases while the number of subjects to test for presence of CV risk factors is smaller. Such an approach facilitates a more effective and simple strategy for risk factor screening.

Keywords: albuminuria; cardiovascular risk factors; screening

Introduction

Cardiovascular disease (CVD) is the most prevalent cause of death in most Western societies. Known risk factors are diabetes, hypertension and hypercholesterolaemia. The presence of these risk factors is frequently undiagnosed. Both US [1–3] and European [4–6] epidemiological data indicate that for every known diabetic, hypertensive or hypercholesterolaemic subject there are one or two subjects with these risk factors yet undiagnosed and thus untreated. As these risk factors can be treated and correction is found to be associated with a better cardiovascular (CV) prognosis, attention focuses on an early detection of these risk factors. Another risk factor, which is also frequently undiagnosed and which presently attracts much attention as cardiovascular risk factor, is impaired glomerular filtration rate (GFR), defined as an eGFR <60 mL/min/1.73 m^2 [stages 3–5 chronic kidney disease (CKD)] [7–9].

To prevent cardiovascular events, guidelines advocate a case-finding approach by screening of individuals for presence of diabetes, hypertension and/or hypercholesterolaemia. However, a case-finding approach is labour and cost intensive, as trained personnel is required to measure blood pressure and to perform venapuncture. Moreover, many subjects have to be screened to find a small proportion positive. These two factors limit the cost effectiveness of such a case-finding screening strategy.

Microalbuminuria has been established as a marker of generalized endothelial damage and is associated with high prevalence of diabetes, hypertension, hypercholesterolaemia and impaired eGFR [10–15]. Microalbuminuria is, moreover, associated with an increased risk for cardiovascular events [16–20]. Microalbuminuria can be assessed from a single-spot morning urine sample [21,22]. Such a sample can be collected at home and sent by post to a central laboratory where albuminuria is measured [13]. Those subjects with an albuminuria level above a certain predefined cut-off value could then be invited for further assessment of cardiovascular risk factors. It has been shown that such an approach is simple and involves low costs [23], making assessment of albuminuria a potentially valuable tool for population screening.

Given the aforementioned considerations, we questioned whether a population screening with pre-selection of subjects based on determination of a urinary albumin concentration (UAC) measured in a first morning void urine sample enhances the efficacy of screening for newly diagnosed cardiovascular risk factors when compared to screening the unselected overall population. We also studied what the cut-off point for defining abnormal UAC should be, and what the benefit is of repeated and more accurate measurements of albuminuria. For these purposes, we used the data obtained in the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study, a prospective, observational cohort study in which subjects collected a first morning urine sample and were subsequently characterized with regard to their cardiovascular risk profile [13].

Materials and methods

Study design and population

This study is part of the PREVEND study, which is designed to prospectively investigate the natural course of albuminuria and its impact on renal and CVD in a large cohort drawn from the general population. Details of this protocol have been described in detail previously [24,25] and can be found at http://www.PREVEND.org. In summary, the subjects of the PREVEND cohort have been selected in 1997 from 40 856 subjects from the general population, aged between 28 and 75 years,

based on their UAC in a morning urine sample. All subjects with a UAC ≥10 mg/L (group A) and a random sample of subjects with a UAC < 10 mg/L (group B) were invited for detailed investigations in an outpatient clinic, including collection of two consecutive 24-h urines. This procedure was chosen to obtain a cohort enriched for the presence of albuminuria, the primary parameter under investigation in the PREVEND study. Of group A, 6000 subjects completed the screening protocol and of group B, 2592 subjects. These 8592 subjects form the PREVEND cohort. Considering that the selection of subjects into the PREVEND cohort is based on UAC (with enrichment for subjects with a UAC >10 mg/L), the prevalence rates of various cardiovascular risk factors may not be comparable with those in the general population. Therefore, we used for this analysis a sub-sample of 3432 subjects that has been formed by reweighing the 'oversampled' group A, thus accounting for the enrichment procedure. These subjects form a representative sample of the general population. Detailed information how reweighing has been achieved has been published previously [22,24]. Since we wanted to study a non-diabetic population, we excluded subjects with self-reported diabetes ($n = 40$). Therefore, our study population finally consisted of 3392 subjects. The PREVEND study was approved by the medical ethics committee of our institution and is conducted according the guidelines of the declaration of Helsinki. All participants who attended the outpatient clinic gave written informed consent.

Measurements

The screening programme in the outpatient clinic consisted of two visits. At the first visit, participants completed a questionnaire regarding demographics, cardiovascular and renal disease history, smoking habits and use of medication for diabetes, and hypertension or hypercholesterolaemia. During both visits, blood pressure was measured with an automatic device (Dinamap XL Model 9300, Johnson-Johnson Medical, Tampa, FL) in supine position during 10 min. Systolic and diastolic blood pressure measurements were calculated as the mean of the last two out of 10 successive Dinamap measurements of the two visits. Furthermore, anthropometric measurements (weight, height and BMI) were performed, blood was drawn in fasting condition and subjects were asked to collect 24 h urine on two consecutive days.

UAC was determined by nephelometry, with a threshold of 2.3 mg/L and intra- and inter-assay coefficients of variation of 2.2 and 2.6%, respectively (BNII, Dade Behring Diagnostic, Marburg, Germany). Measurements of serum creatinine, cholesterol and plasma glucose were determined by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, NY, USA), an automatic enzymatic method.

Definitions

According to the JNC7 and ESH 2003 guidelines, hypertension is defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg or the use of antihypertensive medication [26–28]. Diabetes is defined as fasting glucose levels of ≥7.0 mmol/L, non-fasting glucose levels of ≥11.1 mmol/L or the use of glucose lowering medication in accordance with the recommendations from the American Diabetes Association [29]. Hypercholesterolaemia is defined in case of a previous myocardial infarction as the use of lipid-lowering drugs, or a total serum cholesterol of ≥5.18 mmol/L (≥200 mg/dL) or HDL cholesterol of <1.03 mmol/L (<40 mg/dL) and total serum cholesterol/HDL cholesterol ratio of ≥5. Without a previous myocardial infarction, hypercholesterolaemia is defined as total serum cholesterol of ≥6.20 mmol/L (≥240 mg/dL) or HDL cholesterol of <0.90 mmol/L (<35 mg/dL) and total serum cholesterol/HDL cholesterol ratio of ≥6 [30–33]. A history of CVD is defined as a myocardial infarction or cerebrovascular accident with a hospital admission in the past. Smoking is defined as current smoking or cessation of smoking <1 year before the first visit. GFR was estimated with the modified MDRD formula, taking into account sex, age, race and serum creatinine. An MDRD clearance <60 mL/min/1.73 m^2 is defined as impaired GFR [7, 34]. Urinary albumin excretion (UAE) is given as the average of the two 24 h urine collections. An elevated albuminuria is defined, in case of just the first morning urine void, as a UAC ≥20 mg/L, and in case of two 24 h urine collections, as a UAE ≥30 mg/24 h. Hypertension, hypercholesterolaemia, CKD and elevated albuminuria are considered to be known when the subject involved reported in the questionnaire use of blood pressure or lipid-lowering medication, presence of renal disease or elevated albuminuria, respectively.

Statistical analyses

Analyses were performed using the statistical package SPSS 12.0 (SPSS, Chicago, IL, USA). Continuous data are reported as means with standard deviation. In case of a skewed distribution, medians and interquartile ranges are presented. Prevalences are presented as percentages. Differences between groups for continuous data were tested by Student's *t*-test or a Mann–Whitney rank test in the case of skewed distribution. Differences in prevalence were tested with a χ^2 test. We first tested the number of subjects that were detected to have diabetes, hypertension, hypercholesterolaemia and impaired GFR according to their albumin concentration in the morning urine sample, with special attention to newly detected presence of these CV risk factors. We performed a sensitivity analysis and calculated, respectively, sensitivity, specificity, positive and negative predictive value. In this analysis, we also evaluated the diagnostic yield of a urinary albumin creatinine ratio (UACR). As definition for elevated albuminuria, we used in that analysis a UACR in males of ≥2.0 mg/mmol (≥20 mg/g) and in women of ≥3.0 mg/mmol (≥30 mg/g) [35]. As we previously showed that the number of subjects with proven microalbuminuria (UAE 30–300 mg/24 h) using the traditional cut-off value for presence of microalbuminuria in a spot morning sample (being 20–200 mg/L) will result in a considerable number of false negative results [22], we also analysed the impact of applying a lower cut-off value of 10 mg/L. We next tested whether confirmation of an elevated UAC by additional 24 h urine testing may further improve the diagnostic yield of cardiovascular risk factors.

Results

Mean age of the 3392 subjects was 48 ± 12 years, and the population consisted predominantly of subjects of Caucasian origin (Table 1). In line with the fact that a sample representative of the general population is studied, it appeared that on average blood pressure, serum cholesterol, plasma glucose, eGFR and albuminuria were within the normal range.

In the overall study population, the number of subjects newly diagnosed with either diabetes, hypertension, hypercholesterolaemia and/or impaired eGFR was 3.6-fold higher than the number of subjects known with such a risk factor (1331 versus 370 subjects) (Table 2). The number of subjects with a known as compared to a new diagnosis of a risk factor differed for the individual risk factors. For one subject known with hypertension, two had a new diagnosis of hypertension. These figures were 1:6 for hypercholesterolaemia and 1:30 for impaired eGFR.

The chance for having a risk factor newly diagnosed increased with a higher UAC, especially in the group with a UAC ≥20 mg/L (Table 2). The prevalence of new diabetes, hypertension, hypercholesterolaemia and/or impaired eGFR was higher in the 267 subjects with a UAC ≥20 mg/L compared to the overall population (61.0 versus 39.2%; $P < 0.05$). This was due to the fact that the prevalences of these newly diagnosed risk factors were all higher in the group with a UAC ≥20 mg/L than in the group with a UAC <10 mg/L. Data on sensitivity, specificity, and positive and negative predictive value are given in Table 3. The data show that after confirmation with 24-h urine collections, the sensitivity decreases and specificity increases as expected. Consequently, positive predictive value increases whereas

Table 1. Population characteristics

	Total group
Persons	3392
Age, year	48 ± 12
Male, %	45.2
Caucasian, %	95.4
Smokers, %	41.2
CV history with hospital admission, %	3.0
BMI, kg/m^2	25.8 ± 4.1
Systolic BP, mm Hg	126 ± 18
Diastolic BP, mm Hg	73 ± 9
Antihypertensive drugs, %	8.2
Total cholesterol, mmol/L	5.6 ± 1.1
Lipid-lowering drugs, %	3.9
Plasma glucose, mmol/L	4.7 ± 0.9
Serum creatinine, μmol/L	83 ± 14
eGFR, mL/min	80 ± 14
Urine albumin concentration, mg/L	5.7 (2.3–26.9)

Continuous variables are presented as mean and standard deviation; urinary albumin concentration is presented as median value with 95% confidence interval.
Abbreviations: CV, cardiovascular; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate.

negative predictive value did not change. The sensitivity analysis using UACR sex specific cut-off values showed comparable results.

In the subgroup of 557 subjects with a UAC between 10 and 20 mg/L, the prevalence of any newly diagnosed CV risk factor was comparable with the prevalence in the overall population (39.7 versus 39.2%; $P > 0.05$). This was due to the fact that only the prevalence of newly diagnosed hypertension (and of course of albuminuria) was higher, but not of new diabetes, hypercholesterolaemia and/or impaired GFR when compared to the group with a UAC <10 mg/L.

When we next confirmed the presence of an elevated albumin excretion in two 24 h urine samples, we found about half of the subjects with a UAC ≥20 mg/L to have a UAE ≥30 mg/24 h (Table 4, right columns, 140 out of 267). The overall prevalence of subjects with any newly detected CV risk factor in the group of subjects with at two occasions elevated albuminuria was obviously higher compared to the group that at first measurement had a UAC ≥20 mg/L, but at confirmation appeared not to have an elevated UAE (76.4 versus 44.1%). This was due to a higher prevalence of all individual risk factors in the UAE ≥30 mg/24 h group compared to the UAE <30 mg/24 h group. In fact, the prevalences of the individual risk factors in this group, with only positive UAC but negative UAE value, were not much different compared to that in the overall background population (Table 2, first column). We found that in the group of subjects with a UAC between 10 and 20 mg/L, only a minority (8.3%) had an elevated UAE (≥30 mg/24 h) at confirmation. But in these subjects, the yield of newly diagnosed risk factors was higher than in the subjects with UAC ≥20 mg/L and UAE <30 mg/24 h.

Figure 1 shows the prevalence of newly diagnosed cardiovascular risk factors in the overall (unselected) popula-

Table 2. Classical and renal risk factors for cardiovascular disease in the overall population and when this population is subdivided according to urinary albumin concentration in a single first morning void urine sample

	Total	UAC <10	10≤ UAC <20	UAC ≥20
n	3392 (100%)	2568 (75.7%)	557 (16.4%)	267 (7.9%)
Age, year	48.4 ± 12.3	47.8 ± 12.0	48.4 ± 13.0	53.2 ± 12.9*
Male	(45%)	(43%)	(51%)*	(54%)*
Subjects with any RF				
Newly diagnosed	1331 (39.2%)	947 (36.9%)	221 (39.7%)	163 (61.0%)*
Known	370 (10.9%)	242 (9.4%)	71 (12.7%)*	57 (21.3%)*
Total	1547 (45.6%)	1095 (42.6%)	264 (47.4%)*	188 (70.4%)*
Diabetes				
Newly diagnosed	46 (1.4%)	23 (0.9%)	10 (1.8%)	13 (4.9%)*
Known	NA	NA	NA	NA
Hypertension				
Newly diagnosed	577 (17.0%)	365 (14.2%)	122 (21.9%)*	90 (33.7%)*
Known	279 (8.2%)	185 (7.2%)	47 (8.4%)	47 (17.6%)*
Hypercholesterolaemia				
Newly diagnosed	839 (24.7%)	619 (24.1%)	118 (21.2%)	102 (38.2%)*
Known	132 (3.9%)	86 (3.3%)	30 (5.4%)*	16 (6.0%)*
Impaired GFR				
Newly diagnosed	179 (5.3%)	129 (5.0%)	22 (3.9%)	28 (10.5%)*
Known	5 (0.1%)	4 (0.2%)	0 (0.0%)	1 (0.4%)
Albuminuria				
Newly diagnosed	194 (5.7%)	24 (0.9%)	46 (8.3%)*	124 (46.4%)*
Known	170 (5.0%)	116 (4.5%)	30 (5.4%)	24 (9.0%)*

*$P < 0.05$ vs UAC <10 mg/L.
Variables are expressed as *n* (%). Age is given as mean and SD.
Abbreviations: UAC, urinary albumin concentration (mg/L); RF, risk factor; GFR, glomerular filtration rate; albuminuria, UAE ≥30 mg/24 h; NA, not applicable.

Table 3. Sensitivity analysis of albuminuria screening to detect subjects with classical and renal risk factors for cardiovascular disease in the overall population

	Sensitivity %	Specificity %	PPV %	NPV %
UAC ≥20				
Any risk factor	12	96	70	57
Diabetes	28	92	5	99
Hypertension	16	95	51	77
Hypercholesterolaemia	12	94	44	73
CKD	16	93	11	95
UACR, F ≥3.0 or M ≥2.0				
Any risk factor	10	97	75	55
Diabetes	28	94	6	99
Hypertension	14	96	57	76
Hypercholesterolaemia	10	95	46	72
CKD	15	88	15	95
UAC ≥20 + UAE ≥30				
Any risk factor	8	99	84	56
Diabetes	20	96	6	99
Hypertension	11	98	64	76
Hypercholesterolaemia	8	97	56	73
CKD	13	96	16	95

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; UAC, urinary albumin concentration (mg/L); UACR, urinary albumin creatinine ratio (mg/mmol); UAE, urinary albumin excretion (mg/24 h); CKD, chronic kidney disease.

tion (n = 3392), and in case a selection strategy is followed, first based upon one single first morning urine void in which UAC is ≥20 mg/L and at confirmation the average UAE in two subsequent 24 h urine samples is equal or more than 30 mg (n = 140).

Discussion

In our study cohort, which is representative for the general population, the number of subjects newly diagnosed to have a CV risk factor is 3.6-fold higher than the number known already with a risk factor. Screening the general population for an elevated UAC (≥20 mg/L) in one first morning void urine sample increased the relative prevalence of newly diagnosed risk factors. Further studies on the presence of these risk factors can then be limited to 267 (7.9%) instead of the original 3392 subjects. Although the sensitivity of this pre-screening to detect new CV risk factors is low, its specificity is good. The relative prevalence of newly diagnosed risk factors further improved when we confirmed the elevated albuminuria level via two 24 h urine collections.

The prevalences of albuminuria and of hypertension and hyperlipidaemia in this population-based study is comparable to that in other general population-based cohorts, such as in the recently published CKD prognosis consortium report on the impact of GFR and albuminuria on mortality, including four European and two Asian cohort studies [36]. Our finding that in population screening the number of subjects with a new diagnosis of a risk factor is more than 3-fold higher than the number already known with a risk factor is in line with data from literature [1–6]. In addition

Table 4. The effect of confirmation of findings in a first morning void urine sample by two subsequent 24-h urines for classical and renal risk factors for cardiovascular disease

		10≤ UAC <20		UAC ≥20	
		n= 557 (16.4%)		n= 267 (7.5%)	
	UAC <10	UAE <30	UAE ≥30	UAE <30	UAE ≥30
n	2568 (75.7%)	508 (91.7%)	49 (8.3%)	127 (46.1%)	140 (53.9%)
Age, year	47.8 ± 12.0	47.9 ± 12.9	53.4 ± 13.1	49.3 ± 12.4	56.5 ± 12.4*
Male	(43%)	(51%)*	(51%)	(43%)	(64%)*
Subjects with any RF					
Newly diagnosed	947 (36.9%)	195 (38.4%)	26 (53.1%)*	56 (44.1%)	107 (76.4%)*
Known	242 (9.4%)	58 (11.4%)	13 (26.5%)*	23 (18.1%)*	34 (24.3%)*
Total	1095 (42.6%)	231 (45.5%)	33 (67.3%)*	71 (55.9%)*	117 (83.6%)*
Diabetes					
Newly diagnosed	23 (0.9%)	7 (1.4%)	3 (6.1%)*	4 (3.1%)*	9 (6.4%)*
Known	NA	NA	NA	NA	NA
Hypertension					
Newly diagnosed	365 (14.2%)	105 (20.7%)*	17 (34.7%)*	27 (21.3%)*	63 (45.0%)*
Known	185 (7.2%)	39 (7.7%)	8 (16.3%)*	20 (15.7%)*	27 (19.3%)*
Hypercholesterolaemia					
Newly diagnosed	619 (24.1%)	108 (21.3%)	10 (20.4%)	34 (26.8%)	68 (48.6%)*
Known	86 (3.3%)	24 (4.7%)	6 (12.2%)*	5 (3.9%)	11 (7.9%)*
Impaired GFR					
Newly diagnosed	129 (5.0%)	17 (3.3%)	5 (10.2%)	5 (3.9%)	23 (16.4%)*
Known	4 (0.2%)	0 (0.0%)	0 (0.0%)	1 (0.8%)	0 (0.0%)
Albuminuria					
Newly diagnosed	24 (0.9%)	0 (0.0%)*	46 (93.9%)*	0 (0.0%)	124 (88.6%)*
Known	116 (4.5%)	27 (5.3%)	3 (6.1%)	8 (6.3%)	16 (11.4%)*

*$P < 0.05$ vs UAC <10 mg/L.

Variables are expressed as n (%). Age is given as mean and SD.

Abbreviations: UAC, urinary albumin concentration (mg/L); UAE, urinary albumin excretion (mg/24 h); RF, risk factor; GFR, glomerular filtration rate; albuminuria, UAE ≥30 mg/24 h; NA, not applicable.

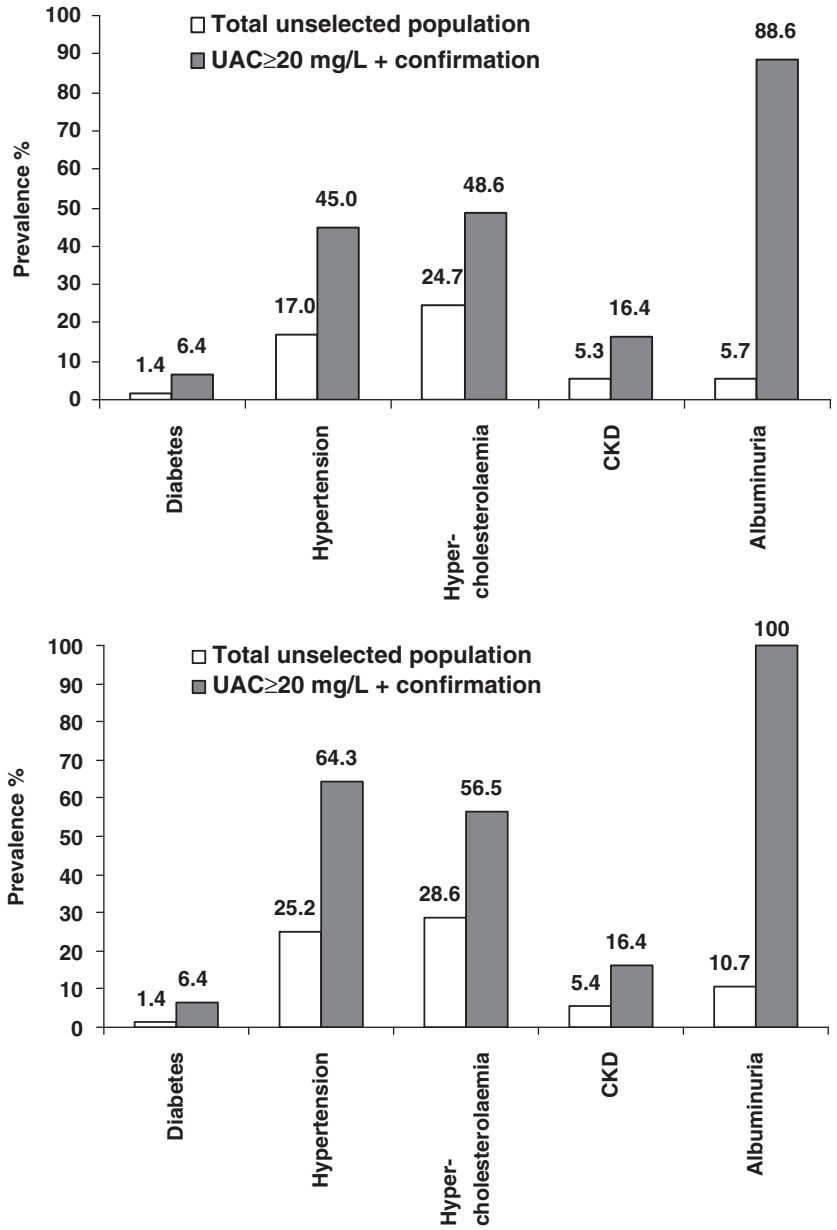

Fig. 1. Prevalence of newly discovered (upper panel) and total (lower panel) classical and renal risk factors for cardiovascular disease in a random sample of the general population. Data are given for the overall, unselected population (open bars) and for the subpopulation with a UAC ≥20 mg/L in a first morning void urine sample and confirmation of elevated albuminuria in two subsequent 24-h urine collections (UAE ≥30 mg/24 h).

to these data, we found that the ratio of new versus known risk factor is higher for hypercholesterolaemia and impaired GFR than for hypertension. That may be due to the more strict definition of hypercholesterolaemia than of hypertension, resulting in a larger number of hypercholesterolaemic than hypertensive patients. It may also reflect that general practitioners, as yet, are screening less for hypercholesterolaemia or are reluctant to diagnose someone as being hypercholesterolaemic. On the other hand, the high ratio for impaired GFR will, in our opinion, be predominantly due to the fact that most subjects with an impaired GFR have not yet been diagnosed. This is a well-known phenomenon in studies evaluating the awareness of CKD [37].

Our finding that subjects with a UAC ≥20 mg/L have a higher prevalence of CV risk factors is also in line with literature. Many risk factors have been found associated with elevated albuminuria, diabetes and hypertension being the most prominent ones, while the association between hypercholesterolaemia and an elevated albuminuria is less well described [38]. An increased albuminuria, of course, may also been found more frequently in subjects with an impaired GFR, although subjects with an elevated GFR have also more frequently an elevated albuminuria [39]. As our present study is cross-sectional in design, we did not document whether subjects with a UAC ≥20 mg/L are also at increased risk to develop diabetes,

hypertension, hypercholesterolaemia or impaired GFR in the years thereafter. Such a phenomenon has, however, also been described for new diabetes [40,41], new hypertension [42,43] and new stage 3 or 4 CKD [44].

In our study, we have chosen for a first screening by measuring UAC in a first morning void urine sample. To that purpose, the subject was sent via post a plastic vial to collect a sample of that urine, which could be sent via post to the central laboratory. If such an approach is not feasible, the urine sample can also be brought to the laboratory or a screening facility. In our setting, we confirmed an elevated albuminuria by measuring the average UAE in two 24 h urine collections. Our present data show, however, that after confirmation only about half of the subjects that originally tested positive in the morning void (UAC ≥20 mg/L) indeed appeared to have an elevated 24 h albuminuria (UAE ≥30 mg/24 h). The data also show that the diagnostic yield for newly diagnosed CV risk factors is also much higher when the elevated albuminuria is confirmed. Collecting 24 h urines is in clinical practice a cumbersome procedure. It can be discussed whether the collection of two additional first morning void urines is sufficient for confirmation that a subject has elevated albuminuria or not. Interestingly, it was recently shown that microalbuminuria status, when diagnosed in first morning void urine samples, was at least as reliable to predict CV events during follow-up, as microalbuminuria status measured in 24 h urine samples [24].

By our selection procedure, we were able to limit the number of subjects to test into more detail (blood pressure, cholesterol, glucose levels and kidney function) from 3392 to 267 (= 7.9%) subjects (based upon one urine sample with an abnormally elevated UAC) or even to 140 (= 4.1%) subjects (based upon one UAC ≥20 mg/L which was confirmed by the average of two 24 h UAE ≥30 mg). This approach improved the diagnostic yield and lowers the work load to further test the subjects to a minimum. Far less subjects will have to visit a screening facility in comparison to when the overall general population is to be screened at such a facility. Such a group will even get smaller when only those subjects with any risk factor, such as smoking, or those subjects above some age are screened.

We recently showed that screening of all adult subjects for CV risk factors, eGFR and accurate 24 h albuminuria excretion costs €44 200 per life year gained to detect and subsequently treat one subject with microalbuminuria. When we use the approach of a simple pre-screening on one single morning urine UAC measurement (as described in the present manuscript) and then limit the detailed screening only to those with a UAC ≥20 mg/L, the costs were much lower: €22 000 per life year gained to detect and treat one subject with microalbuminuria. When limiting the screening to those >60 years of age, it further decreased to €7800 per life year gained [45]. Moreover, it was recently shown that the impact of having low normal, high normal, microalbuminuria or macroalbuminuria for all cause and cardiovascular mortality is identical as the impact of having a negative, a trace positive, a 1+ or a 2+ result on a simple urine protein dipstick test [36]. This shows that alternatives for the UAC or UACR test are also feasible and may even result in better cost-effectiveness data. This may facilitate implementation of such programmes in developing countries.

One might counter argue that the sensitivity of the test to diagnose all unknown diabetes, hypertension, hypercholesterolaemia and impaired GFR was low and only modestly improved when lowering the UAC cut-off value to 10 mg/L. Indeed, we did not diagnose new diabetes in 33 out of the 46 subjects, new hypertension in 487 out of the 577 subjects, new hypercholesterolaemia in 737 out of the 839 subjects and new CKD in 151 out of the 179 subjects. Is this missing of new diagnoses of CV risk factors really an argument against our approach? That, of course, is dependent on the fact on what the CV prognosis is in subjects with that risk factor, but without elevated albuminuria. It is well known that subjects with elevated albuminuria have a significantly worse CV risk than those without elevated albuminuria, independent of what CV risk factor is studied, be it diabetes [16–18, 20], hypertension [16–20,46,47] or hypercholesterolaemia [16,20,47]. It has similarly been shown that subjects with mildly impaired GFR with microalbuminuria have a worse CV and renal risk than those without albuminuria [48–52]. Interestingly, Jensen *et al.* described that subjects that were newly identified with hypertension, but had no microalbuminuria, had a 10-year CV disease incidence of only 5% [53]. Such a low risk does not normally merit preventive treatment. In contrast, those subjects with newly identified hypertension and elevated albuminuria had a high 10-year CV disease incidence, accumulating to 25%. We similarly showed that hypertensive subjects without elevated albuminuria have no significant benefit after start of antihypertensive treatment, while the start of antihypertensives resulted in a significantly better CV survival when the hypertension was associated with an elevated albuminuria [54]. These data taken together suggest that screening, primarily on albuminuria, and subsequently, screening those that are found positive for additional CV risk factors, should identify the CV high-risk subjects especially, whereas on average low-risk subjects that will not benefit from preventive treatment will be missed. Missing of new diagnoses of CV risk factors seems therefore no major argument against this approach.

As it was proposed that the cut-off value to define an elevated albuminuria at pre-screening should be lowered [16,21,22], we also studied whether yet undiagnosed CV risk factors were also more frequent in subjects with a UAC 10–20 mg/L. This subgroup overall, however, only showed significantly more new hypertension and new albuminuria, but not more new diabetes and new impaired GFR than subjects with UAC <10 mg/L (Table 2). Moreover, only a minority of these subjects (49 out of 557 (8.3%), Table 4) appeared after confirmation with two subsequent 24-h urines to have elevated albuminuria. We, therefore, conclude that it has no major benefit to lower the cut-off value defining abnormal albuminuria in a first morning void urine sample at a first screening when one wants to diagnose unknown CV risk factors.

To keep the burden and costs as low as possible in population-based screening for elevated albuminuria, we choose for pre-screening by measuring UAC in a morning

urine sample. In clinical practice, some favour measuring the albumin:creatinine ratio (ACR). We previously compared the diagnostic performance of UAC and ACR, measured in a morning urine sample, in predicting a UAE ≥ 30 mg in two subsequent 24-h urines [22]. The diagnostic performance of measuring UAC was satisfactory and comparable to that of measuring ACR. Jafar *et al.* showed comparable results in Indo-Asians [21]. In accordance, our sensitivity analysis using ACR instead of UAC showed similar results with respect to the identification of previously undiagnosed presence of CV risk factors.

Strengths of our study are that it is performed in a sample that is representative for the general population of community-dwelling subjects and that it has detailed information on disease history, objective measurements of CV risk factors and medication use. Furthermore, in contrast to previous studies, it does not restrict itself to one CV risk factor, but offers an integral approach to all presently acknowledged CV (diabetes, hypertension, hypercholesterolaemia) and renal (impaired eGFR, elevated albuminuria) risk factors. We acknowledge also limitations to this study. Our population consisted of almost only Caucasians. Therefore, our conclusions cannot be extrapolated to other ethnic populations.

In conclusion, population screening for elevated albuminuria (UAC ≥20 mg/L) in a single first morning void urine sample results in identification of a population with a high percentage of previously unknown cardiovascular risk factors. Confirmation of elevated albuminuria status in subsequent urine samples even further enriches the prevalence of these previously unknown risk factors. Prescreening for elevated albuminuria will thus increase the efficacy of case finding for cardiovascular risk factors.

Acknowledgements. We thank Dade Behring (Marburg, Germany) for supplying equipment (Behring Nephelometer II) and reagents for nephelometric measurement of UAC. The PREVEND study has been made possible by grants of the Dutch Kidney Foundation, for which we are grateful. The funding for this study had no role in its design, conduct, analysis or in the decision to submit the study for publication.

Conflict of interest statement. None declared.

References

1. Hadden WC, Harris MI. Prevalence of diagnosed diabetes, undiagnosed diabetes, and impaired glucose tolerance in adults 20-74 years of age. *Vital Health Stat* 1987; 11: 1–55
2. Cowie CC, Rust KF, Byrd-Holt DD *et al*. Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health and Nutrition Examination Survey 1999-2002. *Diabetes Care* 2006; 29: 1263–1268
3. Burt VL, Cutler JA, Higgins M *et al*. Trends in the prevalence, awareness, treatment, and control of hypertension in the adult US population. Data from the health examination surveys, 1960 to 1991. *Hypertension* 1995; 26: 60–69
4. Creagh D, Neilson S, Collins A *et al*. Established cardiovascular disease and CVD risk factors in a primary care population of middle-aged Irish men and women. *Ir Med J* 2002; 95: 298–301
5. Smith WC, Lee AJ, Crombie IK *et al*. Control of blood pressure in Scotland: the rule of halves. *BMJ* 1990; 300: 981–983
6. British family heart study: its design and method, and prevalence of cardiovascular risk factors. Family heart study group. *Br J Gen Pract* 1994; 44: 62–67
7. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; 39: S1–S266
8. Levey AS, Beto JA, Coronado BE *et al*. Controlling the epidemic of cardiovascular disease in chronic renal disease: what do we know? What do we need to learn? Where do we go from here? National Kidney Foundation Task Force on Cardiovascular Disease. *Am J Kidney Dis* 1998; 32: 853–906
9. Go AS, Chertow GM, Fan D *et al*. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004; 351: 1296–1305
10. Atkins RC, Polkinghorne KR, Briganti EM *et al*. Prevalence of albuminuria in Australia: the AusDiab Kidney Study. *Kidney Int Suppl* 2004; S22–S24
11. Joshi VD, Mooppil N, Lim J. Prevalence and risk factors of undetected proteinuria in an elderly South-East Asian population. *Nephrology* 2006; 11: 347–354
12. Yuyun MF, Khaw KT, Luben R *et al*. Microalbuminuria and stroke in a British population: the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) population study. *J Intern Med* 2004; 255: 247–256
13. Hillege HL, Janssen WM, Bak AA *et al*. Microalbuminuria is common, also in a nondiabetic, nonhypertensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. *J Intern Med* 2001; 249: 519–526
14. Pruijm MT, Madeleine G, Riesen WF *et al*. Prevalence of microalbuminuria in the general population of Seychelles and strong association with diabetes and hypertension independent of renal markers. *J Hypertens* 2008; 26: 871–877
15. Choi HS, Ryu SH, Lee KB. The relationship of microalbuminuria with metabolic syndrome. *Nephron Clin Pract* 2006; 104: c85–c93
16. Klausen K, Borch-Johnsen K, Feldt-Rasmussen B. Very low levels of microalbuminuria are associated with increased risk of coronary heart disease and death independently of renal function, hypertension, and diabetes. *Circulation* 2004; 110: 32–35
17. Weir MR. Microalbuminuria and cardiovascular disease. *Clin J Am Soc Nephrol* 2007; 2: 581–590
18. de Zeeuw D, Parving HH, Henning RH. Microalbuminuria as an early marker for cardiovascular disease. *J Am Soc Nephrol* 2006; 17: 2100–2105
19. Pontremoli R, Leoncini G, Ravera M *et al*. Microalbuminuria, cardiovascular, and renal risk in primary hypertension. *J Am Soc Nephrol* 2002; 13: S169–S172
20. Hillege HL, Fidler V, Diercks GF *et al*. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. *Circulation* 2002; 106: 1777–1782
21. Jafar TH, Chaturvedi N, Hatcher J *et al*. Use of albumin creatinine ratio and urine albumin concentration as a screening test for albuminuria in an Indo-Asian population. *Nephrol Dial Transplant* 2007; 22: 2194–2200
22. Gansevoort RT, Verhave JC, Hillege HL *et al*. The validity of screening based on spot morning urine samples to detect subjects with microalbuminuria in the general population. *Kidney Int Suppl* 2005; S28–S35
23. Atthobari J, Asselbergs FW, Boersma C *et al*. Cost-effectiveness of screening for albuminuria with subsequent fosinopril treatment to prevent cardiovascular events: a pharmacoeconomic analysis linked to the prevention of renal and vascular endstage disease (PREVEND) study and the prevention of renal and vascular endstage disease intervention trial (PREVEND IT). *Clin Ther* 2006; 28: 432–444
24. Lambers Heerspink HJ, Brantsma AH, de Zeeuw D *et al*. Albuminuria assessed from first-morning-void urine samples versus 24-hour urine collections as a predictor of cardiovascular morbidity and mortality. *Am J Epidemiol* 2008; 168: 897–905
25. Mahmoodi BK, Gansevoort RT, Veeger NJ *et al*. Microalbuminuria and risk of venous thromboembolism. *JAMA* 2009; 301: 1790–1797
26. Ramsay L, Williams B, Johnston G *et al*. Guidelines for management of hypertension: report of the third working party of the British Hypertension Society. *J Hum Hypertens* 1999; 13: 569–592

27. Chalmers J, MacMahon S, Mancia G *et al*. 1999 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension. Guidelines sub-committee of the World Health Organization. *Clin Exp Hypertens* 1999; 21: 1009–1060
28. Chobanian AV, Bakris GL, Black HR *et al*. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA* 2003; 289: 2560–2572
29. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 2003; 26: S5–S20
30. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001; 285: 2486–2497
31. Pearson TA, Bowlin S, Sigmund WR. Screening for hypercholesterolemia. *Annu Rev Med* 1990; 41: 177–186
32. Pignone MP, Phillips CJ, Atkins D *et al*. Screening and treating adults for lipid disorders. *Am J Prev Med* 2001; 20: 77–89
33. Conroy RM, Pyorala K, Fitzgerald AP *et al*. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur Heart J* 2003; 24: 987–1003
34. Levey AS, Bosch JP, Lewis JB *et al*. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; 130: 461–470
35. Levey AS, Eckardt KU, Tsukamoto Y *et al*. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 2005; 67: 2089–2100
36. Matsushita K, van der Velde M, Astor BC *et al*. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet* 2010; 375: 2073–2081
37. Coresh J, Byrd-Holt D, Astor BC *et al*. Chronic kidney disease awareness, prevalence, and trends among U.S. adults, 1999 to 2000. *J Am Soc Nephrol* 2005; 16: 180–188
38. Jager A, Kostense PJ, Ruhe HG *et al*. Microalbuminuria and peripheral arterial disease are independent predictors of cardiovascular and all-cause mortality, especially among hypertensive subjects: five-year follow-up of the Hoorn Study. *Arterioscler Thromb Vasc Biol* 1999; 19: 617–624
39. Pinto-Sietsma SJ, Janssen WM, Hillege HL *et al*. Urinary albumin excretion is associated with renal functional abnormalities in a non-diabetic population. *J Am Soc Nephrol* 2000; 11: 1882–1888
40. Brantsma AH, Bakker SJ, Hillege HL *et al*. Urinary albumin excretion and its relation with C-reactive protein and the metabolic syndrome in the prediction of type 2 diabetes. *Diabetes Care* 2005; 28: 2525–2530
41. Halimi JM, Bonnet F, Lange C *et al*. Urinary albumin excretion is a risk factor for diabetes mellitus in men, independently of initial metabolic profile and development of insulin resistance. The DESIR Study. *J Hypertens* 2008; 26: 2198–2206
42. Brantsma AH, Bakker SJ, de Zeeuw D *et al*. Urinary albumin excretion as a predictor of the development of hypertension in the general population. *J Am Soc Nephrol* 2006; 17: 331–335
43. Wang TJ, Evans JC, Meigs JB *et al*. Low-grade albuminuria and the risks of hypertension and blood pressure progression. *Circulation* 2005; 111: 1370–1376
44. Verhave JC, Gansevoort RT, Hillege HL *et al*. An elevated urinary albumin excretion predicts de novo development of renal function impairment in the general population. *Kidney Int Suppl* 2004; S18–S21
45. Boersma C, Gansevoort RT, Pechlivanoglou P *et al*. Screen-and-treat strategies for albuminuria to prevent cardiovascular and renal disease: cost-effectiveness of nationwide and targeted interventions based on analysis of cohort data from the Netherlands. *Clin Ther* 2010; 32: 1103–1121
46. Bigazzi R, Bianchi S, Baldari D *et al*. Microalbuminuria predicts cardiovascular events and renal insufficiency in patients with essential hypertension. *J Hypertens* 1998; 16: 1325–1333
47. Borch-Johnsen K, Feldt-Rasmussen B, Strandgaard S *et al*. Urinary albumin excretion. An independent predictor of ischemic heart disease. *Arterioscler Thromb Vasc Biol* 1999; 19: 1992–1997
48. Brantsma AH, Bakker SJ, Hillege HL *et al*. Cardiovascular and renal outcome in subjects with K/DOQI stage 1-3 chronic kidney disease: the importance of urinary albumin excretion. *Nephrol Dial Transplant* 2008; 23: 3851–3858
49. Ishani A, Grandits GA, Grimm RH *et al*. Association of single measurements of dipstick proteinuria, estimated glomerular filtration rate, and hematocrit with 25-year incidence of end-stage renal disease in the multiple risk factor intervention trial. *J Am Soc Nephrol* 2006; 17: 1444–1452
50. Hallan SI, Ritz E, Lydersen S *et al*. Combining GFR and albuminuria to classify CKD improves prediction of ESRD. *J Am Soc Nephrol* 2009; 20: 1069–1077
51. Hallan S, Astor B, Romundstad S *et al*. Association of kidney function and albuminuria with cardiovascular mortality in older vs younger individuals: the HUNT II Study. *Arch Intern Med* 2007; 167: 2490–2496
52. Iseki K, Kinjo K, Iseki C *et al*. Relationship between predicted creatinine clearance and proteinuria and the risk of developing ESRD in Okinawa, Japan. *Am J Kidney Dis* 2004; 44: 806–814
53. Jensen JS, Feldt-Rasmussen B, Strandgaard S *et al*. Arterial hypertension, microalbuminuria, and risk of ischemic heart disease. *Hypertension* 2000; 35: 898–903
54. Boersma C, Postma MJ, Visser ST *et al*. Baseline albuminuria predicts the efficacy of blood pressure-lowering drugs in preventing cardiovascular events. *Br J Clin Pharmacol* 2008; 65: 723–732

Received for publication: 8.2.10; Accepted in revised form: 13.7.10