N-acetyl-β-D-glucosaminidase does not enhance albuminuria's prediction of cardiovascular or all-cause mortality in a low-risk population.

Marit D. Solbu MD PhD[1], Ingrid Toft MD PhD[1,2¶], Maja-Lisa Løchen MD PhD[3], Ellisiv B. Mathiesen MD PhD[2,4], Bjørn O. Eriksen MD, PhD[1,2], Toralf Melsom MD PhD[1,2], Inger Njølstad MD PhD[3], Tom Wilsgaard PhD[3], Trond G. Jenssen MD PhD[2,5].

[1]Section of Nephrology, University Hospital of North Norway, Tromsø; [2]Dept. of Clinical Medicine, [3]Dept. of Community Medicine, Faculty of Health Sciences, UiT The Arctic University of Norway; [4]Dept. of Neurology, University Hospital of North Norway, Tromsø; [5]Oslo University Hospital, Oslo, Norway.

[¶]Deceased

Running title: NAG does not predict CVD

Word count for abstract: 232

Word count for text: 2813

Address for correspondence: Marit D. Solbu, Section of Nephrology, University Hospital of North Norway, N-9038 Tromsø, Norway

Telephone: +4791864376, E-mail: marit.solbu@unn.no

Abstract

Albuminuria is a well-known risk factor for cardiovascular disease and mortality. Recently, also focus on renal tubular dysfunction as a potential risk factor has been growing. The association between the urinary activity of N-acetyl-β-D-glucosaminidase (NAG) and cardiovascular risk has been assessed mostly in cross-sectional studies. We studied the cross-sectional associations between urinary NAG and cardiovascular risk factors, and also the longitudinal associations between NAG, cardiovascular disease and all-cause mortality in a general population. Urinary NAG/creatinine ratio (NAG ratio) and albumin/creatinine ratio (ACR) were measured in 6834 participants of the Tromsø Study in 1994/95. During 15-17 years of follow-up, 958 myocardial infarctions, 726 ischemic strokes and 2358 deaths were registered. In multivariable analyses adjusted for albuminuria and cardiovascular risk factors, a baseline NAG ratio in the highest quartile was associated with an increased risk of myocardial infarction (hazard ratio (HR) 1.43 (95 % confidence interval (CI) 1.16 – 1.76)), ischemic stroke (HR 1.41 (95 % CI 1.10 – 1.80)) and all-cause mortality (HR 1.60 (95 % CI 1.39 – 1.84)). Combined, ACR and NAG ratio above median gave a 48-80% increased risk for the three endpoints. However, NAG ratio did not add significantly to the baseline risk prediction models when assessed by area under the receiver-operating-characteristics curve or net reclassification improvement. In conclusion, the non-significant improvement of risk prediction does not support the clinical use of NAG ratio in cardiovascular risk assessment in a low-risk group.

INTRODUCTION

N-acetyl-β-D-glucosaminidase (NAG) is a lysosomal enzyme that is distributed in various human tissues[1]. With a molecular weight of 130-140 kDa, circulating NAG is not filtered through the glomeruli. In the kidneys, NAG is found predominantly in lysosomes of proximal tubular cells, and the small amount of NAG normally present in the urine is secreted by these cells by exocytosis. NAG is thus exclusively a marker of tubular cell function[2].

Increased urinary NAG activity has been described in various disease states such as heavy metal poisoning and renal diseases[3-7]. Most authors consider increased urinary NAG to be a sensitive marker of tubular injury[8], related to inflammation and oxidative stress. Albumin in the urine is traditionally considered to be of glomerular origin[9], although impaired tubular reabsorption may contribute in the pathogenesis of increased urinary albumin excretion (UAE)[10]. UAE is a well-established risk factor for development of both cardiovascular disease (CVD) and renal disease[11-15]. However, the mechanisms mediating this risk have not yet been fully established[10]. It is conceivable that tubular markers may further explain the linkage between renal dysfunction including UAE and CVD. The relationship between urinary NAG and end-organ damage has been assessed mainly in cross-sectional studies of diabetic patients[16-19] and in hypertension[20]. Prospective studies of the association between urinary NAG excretion and future development of disease are scarce and have only been carried out in patients with diabetes[21, 22] and chronic heart failure[23].

To our knowledge, the role of urinary NAG as a risk factor for myocardial infarction, ischemic stroke and all-cause mortality has never been investigated, neither in patient groups nor in the general population. The aims of the present population-based, prospective observational study were to investigate the relationship between urinary NAG activity and incidence of myocardial infarction, ischemic stroke and all-cause mortality during 15-17 years of follow-

up, and whether any association was independent of UAE or not. In line with this we also aimed to assess the association between markers of renal dysfunction including urinary NAG, traditional cardiovascular risk factors, and chronic inflammation.

RESULTS

Baseline Characteristics

Among the 6834 persons aged 25-84 years included in the present study, 307 (4.5 %) had diabetes. Altogether 568 (8.3 %) had previous myocardial infarction (n=407) and/or ischemic stroke (n=196). Only 2.1 % (n=146) of the study population had estimated GFR <60 ml/min/1.73m^2, whereas 7.5 % (n=512) had microalbuminuria. Baseline characteristics showed a higher percentage of current smokers and previous myocardial infarction and ischemic stroke among men than among women (Table 1). There was no significant gender difference in the prevalence of diabetes. Urinary NAG concentration was higher in men than in women, but the NAG/creatinine ratio did not differ between the genders. Thus, this parameter was used in the statistical models presented and is named "NAG ratio" in the present paper.

Distribution of Urinary NAG ratio by Age and Comorbidity

The correlation coefficient between urinary albumin/creatinine ratio (ACR) and NAG ratio was 0.31 for women, 0.38 for men and 0.35 for the whole cohort (P<0.001 for all correlations). In unadjusted analyses, urinary NAG ratio increased linearly with age (Table 2). When stratified by quartiles of NAG ratio, cardiovascular risk profile worsened with increasing NAG ratio (Table 3). With the exception of HDL cholesterol, all variables listed

showed an approximately linear association with increasing NAG ratio quartiles. Age adjustment did not change these associations, except for total cholesterol, where the linear group difference disappeared, and for estimated GFR, which demonstrated a weak, but statistically significant, linearity in the opposite direction with age adjustment.

Clinical Events

During a median observation time of 15.3 years, 958 first-ever non-fatal or fatal myocardial infarctions and 726 first-ever non-fatal or fatal ischemic strokes were registered. For all-cause mortality, the median follow-up time was 17.5 years, and 2358 participants (34.5 %) died.

Associations between NAG Ratio, ACR and Event Rate

Crude incidence rates of the three endpoints according to NAG ratio quartiles are shown in Table 4A-C. Increasing rates of both cardiovascular events and mortality of any cause were observed with increasing NAG ratio, and the rates were significantly higher even in the second lowest NAG ratio quartile, compared to the lowest quartile.

Adjusted for age and gender only, NAG ratio was associated with an increased risk of first-ever myocardial infarction, first-ever ischemic stroke and all-cause (data not shown). In multivariable adjusted Cox proportional hazard regression analyses (Table 5A-C), NAG ratio was entered either as a continuous variable (Model 1) or as indicator variables in quartiles with the lowest quartile as reference (Model 2). All models were adjusted for age and gender, cardiovascular risk factors, previous CVD, inflammation (high sensitive C-reactive protein (hsCRP)) and renal biomarkers (log ACR and estimated GFR). As a continuous variable,

NAG ratio did not independently predict ischemic stroke, but the upper quartile of NAG-ratio was associated with a 41% increased risk of stroke compared to the lowest quartile. Both as a continuous variable and categorized, NAG ratio was an independent predictor of myocardial infarction and all-cause mortality, and for all-cause mortality even the second quartile of NAG ratio was associated with a 24% increased risk. There was a significant interaction between gender and NAG ratio for the prediction of myocardial infarction, with a stronger association between the 4th NAG ratio quartile and this endpoint in women than in men, but with a significant association between the 3rd quartile of NAG ratio and myocardial infarction in men only. Significant interaction between gender and NAG ratio was not found for the other endpoints, and there were no interactions with age. In the multivariable models, ACR and hsCRP were significantly associated with all three endpoints, but when hsCRP was removed from the models, the estimates were practically unchanged. Estimated GFR was not associated with any outcome.

There was no statistically significant interaction between NAG ratio and ACR for the association with any endpoint, but the associations between NAG ratio and the endpoints were weakened when adjusted for ACR. However, as the associations were still significant, we explored the composite associations between ACR, NAG ratio and the endpoints.

Adjusted for the same covariates, the combination of ACR above median and/or NAG ratio above median was entered into the model as three indicator variables for the four possible categories. The category with both variables below median was the reference. Table 6A-C shows that individually, ACR or NAG ratio above median gave an increased risk for all-cause mortality of 31-36%, but the combination of high ACR and high NAG ratio was associated with an 80% increased mortality risk compared to reference. The risk for myocardial infarction and ischemic stroke was also 48-79% increased by the combination of ACR and

NAG ratio above median, whereas the associations between any of these entities alone and the cardiovascular endpoints yielded variable results, as shown in detail in Table 6A-B. Among those who experienced each event, the mean number of months from baseline to the event of interest decreased with increasing risk group. For all endpoints, the group with both ACR and NAG ratio above median had the shortest mean time to event.

Discrimination and Reclassification

Area under the receiver-operating-characteristic (ROC) curves (AUC) achieved by three models (Model 1c-3c) are displayed in Table 7. Age, gender and cardiovascular risk factors were included in all models. Estimated GFR and ACR in quartiles were added to Model 2c, and NAG ratio quartiles were finally included in Model 3c. The AUCs for Model 1c were 0.706-0.818 for the three endpoints. For myocardial infarction, Model 3c, but not Model 2c, gave a significantly larger AUC than the baseline model. Both Models 2c and 3c gave AUCs for the prediction of stroke that were significantly larger than Model 1c. Similarly, for all-cause mortality both Models 2c and 3c gave significant increases in the AUC compared to the baseline model, and AUC was significantly larger when derived from Model 3c compared to Model 2c for this endpoint. Net Reclassification Improvement (NRI) by adding ACR and estimated GFR in quartiles (Model 2c) to the baseline model was borderline significant (P=0.08) for the prediction of stroke, but the addition of the NAG ratio quartiles to the model did not further improve the prediction of stroke. NRIs for the other endpoints were not significant when any of the renal biomarkers were added to the baseline model.

DISCUSSION

To our knowledge, this is the first study to describe the association between urinary NAG/creatinine ratio and cardiovascular endpoints as well as all-cause mortality in a general population. The associations with myocardial infarction, ischemic stroke and all-cause mortality were independent of well-known cardiovascular risk factors, increased ACR and reduced estimated GFR when assessed with standard risk metrics. Albuminuria and NAG excretion had an additive predictive effect on all end-points, with an 80% increased relative risk of all-cause mortality and ischemic stroke when both ACR and NAG ratio were above the median value. NAG ratio gave a statistically significant increase in the AUC of the ROC curve for the prediction of all-cause mortality when compared to the AUC achieved from traditional cardiovascular risk factors, estimated GFR and ACR. However, this increase was small and of no clinical significance. Furthermore, the addition of NAG ratio to the models that included estimated GFR and ACR did not increase the AUCs for the prediction of myocardial infarction or stroke, and none of the renal biomarkers gave a significant improvement in risk prediction of any endpoint when quantified using NRI. Therefore, in this low-risk population, NAG ratio lacked important properties to serve as a clinically useful biomarker of cardiovascular risk.

Previous studies on urinary NAG have mainly focused on NAG as a diagnostic and prognostic marker in patients with acute kidney injury[5, 24, 25]. Urinary NAG as a prognostic marker in persons with chronic disease has been studied in patients with heart failure[23, 26, 27] and diabetes[21]. In two clinical studies of patients with chronic heart failure and apparently normal kidney function, urinary NAG was associated with left ventricular dysfunction and predicted all-cause mortality and rehospitalisation for congestive heart failure[23, 27]. Furthermore, urinary NAG was associated with incident myocardial infarction and peripheral

vascular disease during 7 years of follow-up in 124 patients with type 2 diabetes, with risk estimates comparable to UAE[21]. None of these studies applied statistical methods to quantify the improvement in risk prediction by adding NAG to a baseline model.

Recently, a modest association was reported between another urinary tubular marker, Kidney Injury Molecule 1 (KIM-1), and all-cause mortality in participants of the Health, Aging and Body Composition Study. The association was independent of ACR, but a similar association between KIM-1 and CVD was not found[28]. These findings are in line with the results of the present study, which evaluated NAG ratio as the biomarker of tubular dysfunction in a low-risk population; there was a statistically significant relative risk increase of cardiovascular endpoints and death with increasing NAG ratio, but absolute risk prediction was not improved. However, despite the resemblance between the results of the present study and the Health, Aging and Body Composition Study[28], the clinical usefulness of other markers of tubular injury or function is not precluded by these studies. Furthermore, the present results should not be extrapolated to other risk groups and patient populations. In a clinical setting, the change in risk factors and biomarkers may be of importance, and studies including trajectory of a biomarker should be conducted before a biomarker is discarded[29].

It is known that chronically reduced estimated GFR and/or even slightly raised levels of UAE are associated with an increased risk of mortality and CVD, both in patient groups and in the general population[11, 13-15]. In the present study, however, estimated GFR was not associated with any endpoint, perhaps due to the narrow distribution of estimated GFR within the normal range in a relatively healthy population[12, 30].

Notwithstanding the lack of improved risk prediction exhibited by NAG ratio, the independent significant associations between NAG ratio and clinical events may shed some light upon possible pathophysiological processes linking renal dysfunction and CVD, and may thus encourage further research focused on these issues. Albuminuria is considered to be the result of generalized endothelial dysfunction[31] and is shown to co-exist with cardiovascular risk factors such as higher age, obesity, hypertension, early disturbances in the glucose metabolism and diabetes[32-35]. In the present study, increased NAG ratio was found in the same risk groups, which is in line with the existing literature[20, 22, 36-41]. The associations with the endpoints may therefore in part be explained by the more adverse risk profile among individuals with increased NAG ratio. However, since the associations were independent from the traditional cardiovascular risk factors, ACR, and hsCRP, explanations other than NAG ratio solely reflecting an adverse risk profile and inflammation should be sought. . There is evidence that chronic tubulointerstitial hypoxia and increased oxidative stress may play a pathogenetic role in early stages of CKD[42], which in turn is related to the development of CVD. In a large general population cohort participating in the Prevention of Renal and Vascular End Stage Disease (PREVEND) study it was recently shown that compared to matched controls, participants with progressing albuminuria had higher baseline levels of glomerular markers, but lower levels of proximal tubular markers, including urinary NAG[43]. How these observations should be interpreted in view of the present study results, is not intuitively clear, and the relationships between albuminuria, increases in urinary NAG activity, common cardiovascular risk factors, and the development of CVD, may be complicated. Elevations of UAE and NAG in the urine may in part reflect different pathogenetic pathways leading to CVD and death.

Strengths of the present study include the prospective design, a large cohort, a long follow-up and a large number of events. The attendance rate was high and the endpoints were thoroughly validated. Concentrations of albumin[44] and NAG[45] may be falsely and unpredictably low after storage of urine. One of the main strengths of the present study is therefore that ACR and NAG were measured in unfrozen urine specimens at the time of sampling (1994/95). The impact of a day-to-day variation was reduced using the mean values of three and two specimens, respectively.

Limitations include the lack of fasting blood tests. The population included in the Tromsø Study consists almost exclusively of Caucasian middle-aged and elderly persons, limiting broad generalizations. Explicit inferences about causality should not be done from an observational study. Furthermore, all quantitative risk improvement tools have their limitations[29]. Using increases in AUC of ROC curves to evaluate new biomarkers is hampered by the small change observed when even a good biomarker is added to a risk prediction model with a large baseline AUC. The results of the NRI models may have been influenced by the fact the risk classes were arbitrarily chosen. However, the very low NRI values obtained in the present study makes it unlikely that a different risk classification would have changed these results essentially.

In conclusion, in the present population-based study with a long-term follow-up, urinary NAG ratio and ACR were independently associated with first-ever myocardial infarction, first-ever ischemic stroke and all-cause mortality when assessed by standard risk metrics. However, c-statistics and NRI revealed that NAG ratio did not add to the risk predicted by traditional cardiovascular risk factors, estimated GFR and ACR, and therefore does not seem to be a

clinically useful biomarker in low-risk populations. Similar studies assessing the characteristics of other urinary biomarkers of tubular dysfunction should be encouraged.

CONCISE METHODS

Study Population

The Tromsø Study is a population-based, prospective study of inhabitants of the municipality of Tromsø, Northern Norway[46]. In the 4th Tromsø survey (1994/95) all inhabitants in Tromsø born before 1970, were invited to participate, and 27 158 (77%) of eligible subjects attended. All participants aged 55 – 74 years and 5 – 10% of the other birth cohorts (n=9057) were invited to undergo an extensive examination, and the attendance rate for this second visit was 76% (n=6862). Urinary NAG measurements were available in 6834 participants (3369 men and 3465 women). The survey was conducted by the University of Tromsø in cooperation with The National Health Screening Service. The Regional Committee for Medical and Health Research Ethics approved the study, and all participants gave their written consent.

Measurements

The participants returned a self-administered questionnaire, which included information about current medication, presence of diabetes, CVD, smoking habits and physical activity. Leisure physical activity was dichotomized as active (≥1 hour physical activity with prominent perspiration or breathlessness per week), or inactive (all others). Tobacco use was dichotomized into current smokers or not (all others). Blood pressure was recorded in triplet, using an automatic device (Dinamap Vital Signs Monitor1846 Critikon), and the mean of the

second and third readings was used in the analyses. Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic pressure ≥90 mm Hg and/or current use of antihypertensive medication. Height and weight were measured, and body mass index was calculated (kg/m^2).

Urinary NAG and albumin excretion were measured as indicators of proximal tubular and glomerular dysfunction, respectively. Morning urine samples collected on three consecutive days were used. Whereas albumin and creatinine were measured in all three urine samples, NAG was measured in the samples collected on day 1 and 3. A colorimetric method (with 3-cresolsulfonphthaleinyl-N-acetyl-β-D-glucosaminide, Boehringer Mannheim, Germany) was applied for the NAG measurements. Urine albumin and creatinine were analysed with kits from ABX Diagnostics; Montpellier, France. UAE was reported as the mean value of ACR (mg/mmol) in the urine samples collected on day 1, 2 and 3. NAG excretion was given as the mean value of urinary NAG activity (U/L) and NAG/creatinine ratio ("NAG ratio", in U/g Cr) in the specimens collected on day 1 and 3. All measurements were performed in fresh urine samples at the time of collection in 1994/95, thus none of the samples had been frozen and thawed. Microalbuminuria was defined as ACR ≥3.39 mg/mmol.

A non-fasting blood sample was obtained. Plasma creatinine was originally analysed by a modified Jaffe reaction. In 2006, 111 plasma samples from the 1994/95 survey were thawed and reanalysed with an enzymatic method (Modular P/Roche). Creatinine data were fitted to a linear regression model. Recalibrated creatinine values were calculated for all participants and recalculated values were used in the analyses. Creatinine-based estimated GFR was calculated applying the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation[47]. HsCRP was measured in 2007 in serum samples stored at -70°C. Presence of diabetes was

defined as self-reported diabetes mellitus, self-reported use of antidiabetic medication, or HbA1c ≥6.5% or non-fasting plasma glucose >10.0 mmol/L.

Outcomes

Data on cardiovascular events were obtained from the Tromsø Study Cardiovascular Disease Registry. Cardiovascular endpoints were defined as first-ever non-fatal or fatal myocardial infarction and first-ever non-fatal or fatal ischemic stroke. Adjudication of hospitalized and out-of-hospital events was performed by an independent endpoint committee. Event ascertainment followed a detailed protocol, according to established diagnostic criteria[48, 49], based on medical records, autopsy records, and death certificates. Each case was reviewed separately by trained physicians who were blinded for baseline data. The cardiovascular endpoint registry was complete until December 31st, 2010. Individuals who died or emigrated were identified through the Population Registry of Norway. Follow-up time was assigned from the date of screening until the date of the first event or the date of censoring (death from causes other than the event of interest, migration, emigration or end of follow-up, December 31st, 2010). Data on all-cause mortality were obtained from the National Death Registry at Statistics Norway. The national 11-digit identification number allowed a linkage to the Norwegian Population Registry and ensured a complete follow-up status for all-cause mortality until December 31st, 2012. Persons who emigrated were censored on the date of emigration.

Statistical Analysis

Data are presented as mean ± standard deviation (SD) (normally distributed variables), or as median and interquartile range (variables with a skewed distribution). Gender differences within baseline variables were assessed using two-tailed independent samples t-tests, Mann-

Whitney's U-test or chi square tests, as appropriate. Because ACR has a markedly skewed distribution, Spearman's test was used for correlation between NAG ratio and ACR. Kruskall-Wallis test and Jonckheere-Terpstra trend test were used for comparison of NAG ratio and ACR between age groups. For comparison of baseline characteristics across increasing NAG ratio quartiles, one-way ANOVA and Jonckheere-Terpstra trend test were used for continuous and chi square test with linear trend test for categorical variables. Crude incidence rates were calculated as events per 1000 person years at risk. Differences in incidence rates across quartiles of NAG ratio were tested for by normal test with continuity correction. Multivariable Cox proportional-hazard models were applied to examine associations of NAG ratio with first-ever fatal or non-fatal myocardial infarction, ischemic stroke, and all-cause mortality. Covariates were age and gender, comorbidity (diabetes, previous myocardial infarction or ischemic stroke), known cardiovascular risk factors (systolic blood pressure, body mass index, total cholesterol, HDL cholesterol, hsCRP, current smoking, physical activity and the use of antihypertensive drugs), and other markers of renal dysfunction (ACR, estimated GFR). NAG ratio was entered into separate models as a continuous variable and categorized into quartiles. ACR was log transformed to get the best fit of the model.

ACR and NAG ratio were combined into four categories defined by the presence of ACR value above median, NAG ratio above median, none of these or both. HRs and 95% CIs were calculated for this categorization using the absence of both ACR and NAG ratio above median as reference. In these models, the above mentioned covariates were included. Also, the mean (±SD) time to event for those who experienced the event within each of these groups was calculated, and linear trend was tested for using one-way ANOVA. In all Cox regression models, the proportional hazard assumption was checked by visual inspection of the -log-log survival curves. In order to evaluate the added value of ACR and NAG ratio in risk prediction, c-statistics were applied to estimate AUC of ROC curves. Predicted risk for

each endpoint was assessed with logistic regression analyses, using three different models. In Model 1c the above listed cardiovascular risk factors were included, whereas the quartiles of ACR and estimated GFR were added to the baseline model in Model 2c. Model 3c included all variables in Model 2c plus the quartiles of NAG ratio. As suggested by Pencina et al[50] we also calculated the Net Reclassification Improvement (NRI). The predicted probabilities for each endpoint achieved from the logistic regression analyses were categorized into tertiles, and reclassification tables for participants who did and did not experience the endpoints, as predicted by Model 1c, 2c and 3c, were made. NRI_{yes} was calculated as the proportion of participants who experienced the endpoint of interest who were classified to a higher risk group minus the proportion of participants who were classified to a lower risk group by Model 2c and 3c compared to Model 1c. Similarly NRI_{no} was calculated for participants who did not reach an endpoint. The $NRI_{overall}$ was the sum of NRI_{yes} and NRI_{no} for each endpoint. P-values <0.05 were considered statistically significant. Analyses were run using IBM SPSS Statistics software version 22 (IBM Corporation, New York) or SAS software 9.4 (SAS Institute Inc., Cary, NC, USA).

Acknowledgements

The present study was supported financially by the Northern Norway Regional Health Authority.

Statement of Competing Financial Interests

None.

References

1. Skalova, S: The diagnostic role of urinary N-acetyl-beta-D-glucosaminidase (NAG) activity in the detection of renal tubular impairment. *Acta Medica (Hradec Kralove),* 48**:** 75-80, 2005.
2. Bosomworth, MP, Aparicio, SR, Hay, AW: Urine N-acetyl-beta-D-glucosaminidase--a marker of tubular damage? *Nephrol Dial Transplant,* 14**:** 620-626, 1999.
3. Moriguchi, J, Inoue, Y, Kamiyama, S, Horiguchi, M, Murata, K, Sakuragi, S, Fukui, Y, Ohashi, F, Ikeda, M: N-acetyl-beta-D-glucosaminidase (NAG) as the most sensitive marker of tubular dysfunction for monitoring residents in non-polluted areas. *Toxicol Lett,* 190**:** 1-8, 2009.
4. Shelley, R, Kim, NS, Parsons, P, Lee, BK, Jaar, B, Fadrowski, J, Agnew, J, Matanoski, GM, Schwartz, BS, Steuerwald, A, Todd, A, Simon, D, Weaver, VM: Associations of multiple metals with kidney outcomes in lead workers. *Occup Environ Med,* 69**:** 727-735, 2012.
5. Martensson, J, Martling, CR, Bell, M: Novel biomarkers of acute kidney injury and failure: clinical applicability. *Br J Anaesth,* 109**:** 843-850, 2012.
6. Park, HC, Hwang, JH, Kang, AY, Ro, H, Kim, MG, An, JN, In, PJ, Kim, SH, Yang, J, Oh, YK, Oh, KH, Noh, JW, Cheong, HI, Hwang, YH, Ahn, C: Urinary N-acetyl-beta-D glucosaminidase as a surrogate marker for renal function in autosomal dominant polycystic kidney disease: 1 year prospective cohort study. *BMC Nephrol,* 13**:** 93, 2012.
7. Bazzi, C, Petrini, C, Rizza, V, Arrigo, G, Napodano, P, Paparella, M, D'Amico, G: Urinary N-acetyl-beta-glucosaminidase excretion is a marker of tubular cell dysfunction and a predictor of outcome in primary glomerulonephritis. *Nephrol Dial Transplant,* 17**:** 1890-1896, 2002.
8. Siew, ED, Ware, LB, Ikizler, TA: Biological markers of acute kidney injury. *J Am Soc Nephrol,* 22**:** 810-820, 2011.
9. Satchell, SC, Tooke, JE: What is the mechanism of microalbuminuria in diabetes: a role for the glomerular endothelium? *Diabetologia,* 51**:** 714-725, 2008.
10. Dickson, LE, Wagner, MC, Sandoval, RM, Molitoris, BA: The Proximal Tubule and Albuminuria: Really! *J Am Soc Nephrol,* 25**:** 443-453, 2014.
11. Astor, BC, Matsushita, K, Gansevoort, RT, van der Velde, M, Woodward, M, Levey, AS, Jong, PE, Coresh, J, Chronic Kidney Disease Prognosis, C, Astor, BC, Matsushita, K, Gansevoort, RT, van der Velde, M, Woodward, M, Levey, AS, de Jong, PE, Coresh, J, El-Nahas, M, Eckardt, KU, Kasiske, BL, Wright, J, Appel, L, Greene, T, Levin, A, Djurdjev, O, Wheeler, DC, Landray, MJ, Townend, JN, Emberson, J, Clark, LE, Macleod, A, Marks, A, Ali, T, Fluck, N, Prescott, G, Smith, DH, Weinstein, JR, Johnson, ES, Thorp, ML, Wetzels, JF, Blankestijn, PJ, van Zuilen, AD, Menon, V, Sarnak, M, Beck, G, Kronenberg, F, Kollerits, B, Froissart, M, Stengel, B, Metzger, M, Remuzzi, G, Ruggenenti, P, Perna, A, Heerspink, HJ, Brenner, B, de Zeeuw, D, Rossing, P, Parving, HH, Auguste, P, Veldhuis, K, Wang, Y, Camarata, L, Thomas, B, Manley, T: Lower estimated glomerular filtration rate and higher albuminuria are associated with mortality and end-stage renal disease. A collaborative meta-analysis of kidney disease population cohorts. *Kidney Int,* 79**:** 1331-1340, 2011.
12. Solbu, MD, Kronborg, J, Jenssen, TG, Njolstad, I, Lochen, ML, Mathiesen, EB, Wilsgaard, T, Eriksen, BO, Toft, I: Albuminuria, metabolic syndrome and the risk of mortality and cardiovascular events. *Atherosclerosis,* 204**:** 503-508, 2009.
13. Gansevoort, RT, Matsushita, K, van, d, V, Astor, BC, Woodward, M, Levey, AS, de Jong, PE, Coresh, J: Lower estimated GFR and higher albuminuria are associated with adverse kidney outcomes. A collaborative meta-analysis of general and high-risk population cohorts. *Kidney Int,* 80**:** 93-104, 2011.

14. Fox, CS, Matsushita, K, Woodward, M, Bilo, HJ, Chalmers, J, Heerspink, HJ, Lee, BJ, Perkins, RM, Rossing, P, Sairenchi, T, Tonelli, M, Vassalotti, JA, Yamagishi, K, Coresh, J, de Jong, PE, Wen, CP, Nelson, RG: Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without diabetes: a meta-analysis. *Lancet,* 380**:** 1662-1673, 2012.
15. Hallan, SI, Matsushita, K, Sang, Y, Mahmoodi, BK, Black, C, Ishani, A, Kleefstra, N, Naimark, D, Roderick, P, Tonelli, M, Wetzels, JF, Astor, BC, Gansevoort, RT, Levin, A, Wen, CP, Coresh, J: Age and association of kidney measures with mortality and end-stage renal disease. *JAMA*, 308**:** 2349-2360, 2012.
16. Aoki, Y, Yanagisawa, Y, Oguchi, H, Furuta, S: Contribution of hyperglycemia and renal damage to urinary C-peptide clearance in non-insulin-dependent diabetic patients. *Diabetes Res Clin Pract,* 14**:** 85-89, 1991.
17. Hong, CY, Chia, KS, Ling, SL: Urinary protein excretion in Type 2 diabetes with complications. *J Diabetes Complications,* 14**:** 259-265, 2000.
18. Mocan, Z, Erem, C, Yildirim, M, Telatar, M, Deger, O: Urinary beta 2-microglobulin levels and urinary N-acetyl-beta-D-glucosaminidase enzyme activities in early diagnosis of non-insulin-dependent diabetes mellitus nephropathy. *Diabetes Res,* 26**:** 101-107, 1994.
19. Yoshikawa, R, Wada, J, Seiki, K, Matsuoka, T, Miyamoto, S, Takahashi, K, Ota, S, Taniai, K, Hida, K, Yamakado, M, Shikata, K, Uehara, Y, Urade, Y, Makino, H: Urinary PGDS levels are associated with vascular injury in type 2 diabetes patients. *Diabetes Res Clin Pract,* 76**:** 358-367, 2007.
20. Agirbasli, M, Radhakrishnamurthy, B, Jiang, X, Bao, W, Berenson, GS: Urinary N-acetyl-beta-D-glucosaminidase changes in relation to age, sex, race, and diastolic and systolic blood pressure in a young adult biracial population. The Bogalusa Heart Study. *Am J Hypertens,* 9**:** 157-161, 1996.
21. Weitgasser, R, Schnoell, F, Gappmayer, B, Kartnig, I: Prospective evaluation of urinary N-acetyl-beta-D-glucosaminidase with respect to macrovascular disease in elderly type 2 diabetic patients. *Diabetes Care,* 22**:** 1882-1886, 1999.
22. Kern, EF, Erhard, P, Sun, W, Genuth, S, Weiss, MF: Early urinary markers of diabetic kidney disease: a nested case-control study from the Diabetes Control and Complications Trial (DCCT). *Am J Kidney Dis,* 55**:** 824-834, 2010.
23. Damman, K, Masson, S, Hillege, HL, Maggioni, AP, Voors, AA, Opasich, C, Van Veldhuisen, DJ, Montagna, L, Cosmi, F, Tognoni, G, Tavazzi, L, Latini, R: Clinical outcome of renal tubular damage in chronic heart failure. *Eur Heart J,* 32**:** 2705-2712, 2011.
24. Coca, SG, Yalavarthy, R, Concato, J, Parikh, CR: Biomarkers for the diagnosis and risk stratification of acute kidney injury: a systematic review. *Kidney Int,* 73**:** 1008-1016, 2008.
25. Lisowska-Myjak, B: Serum and urinary biomarkers of acute kidney injury. *Blood Purif,* 29**:** 357-365, 2010.
26. Damman, K, Van Veldhuisen, DJ, Navis, G, Vaidya, VS, Smilde, TD, Westenbrink, BD, Bonventre, JV, Voors, AA, Hillege, HL: Tubular damage in chronic systolic heart failure is associated with reduced survival independent of glomerular filtration rate. *Heart,* 96**:** 1297-1302, 2010.
27. Jungbauer, CG, Birner, C, Jung, B, Buchner, S, Lubnow, M, von, BC, Endemann, D, Banas, B, Mack, M, Boger, CA, Riegger, G, Luchner, A: Kidney injury molecule-1 and N-acetyl-beta-D-glucosaminidase in chronic heart failure: possible biomarkers of cardiorenal syndrome. *Eur J Heart Fail,* 13**:** 1104-1110, 2011.

28. Sarnak, MJ, Katz, R, Newman, A, Harris, T, Peralta, CA, Devarajan, P, Bennett, MR, Fried, L, Ix, JH, Satterfield, S, Simonsick, EM, Parikh, CR, Shlipak, MG: Association of Urinary Injury Biomarkers with Mortality and Cardiovascular Events. *J Am Soc Nephrol*, 2014.
29. Pencina, MJ, D'Agostino, RB, Vasan, RS: Statistical methods for assessment of added usefulness of new biomarkers. *Clin Chem Lab Med,* 48**:** 1703-1711, 2010.
30. Toft, I, Solbu, M, Kronborg, J, Mathisen, UD, Eriksen, BO, Storhaug, H, Melsom, T, Lochen, ML, Mathiesen, EB, Njolstad, I, Wilsgaard, T, Brox, J: Cystatin C as risk factor for cardiovascular events and all-cause mortality in the general population. The Tromso Study. *Nephrol Dial Transplant,* 27**:** 2780-2787, 2012.
31. Satoh, M: Endothelial dysfunction as an underlying pathophysiological condition of chronic kidney disease. *Clin Exp Nephrol,* 16**:** 518-521, 2012.
32. Duran-Salgado, MB, Rubio-Guerra, AF: Diabetic nephropathy and inflammation. *World J Diabetes,* 5**:** 393-398, 2014.
33. Klausen, KP, Parving, HH, Scharling, H, Jensen, JS: Microalbuminuria and obesity: impact on cardiovascular disease and mortality. *Clin Endocrinol,* 71**:** 40-45, 2009.
34. Redon, J, Pascual, JM: Development of microalbuminuria in essential hypertension. *Curr Hypertens Rep,* 8**:** 171-177, 2006.
35. Solbu, MD, Kronborg, J, Eriksen, BO, Jenssen, TG, Toft, I: Cardiovascular risk-factors predict progression of urinary albumin-excretion in a general, non-diabetic population: a gender-specific follow-up study. *Atherosclerosis,* 201**:** 398-406, 2008.
36. UK Prospective Diabetes Study (UKPDS). IX: Relationships of urinary albumin and N-acetylglucosaminidase to glycaemia and hypertension at diagnosis of type 2 (non-insulin-dependent) diabetes mellitus and after 3 months diet therapy. *Diabetologia,* 36**:** 835-842, 1993.
37. Girisgen, I, Sonmez, F, Yenisey, C, Kurt-Omurlu, I: Urinary markers of renal damage in hypertensive children diagnosed with ambulatory blood pressure monitoring. *Turk J Pediatr,* 56**:** 48-55, 2014.
38. Goknar, N, Oktem, F, Ozgen, IT, Torun, E, Kucukkoc, M, Demir, AD, Cesur, Y: Determination of early urinary renal injury markers in obese children. *Pediatr Nephrol*, 2014.
39. Ouchi, M, Oba, K, Motoyama, M, Tsunoda, M, Yamashita, H, Aoyama, J, Saigusa, T, Ishii, K, Yano, H, Hashimoto, M, Sekimizu, K, Suzuki, T, Nakano, H: Postprandial glycemic control conditions in relation to urinary N-acetyl-beta-D-glucosaminidase in patients with type 2 diabetes mellitus without low glomerular filtration rate. *Diabetes Technol Ther,* 16**:** 41-47, 2014.
40. Ouchi, M, Suzuki, T, Hashimoto, M, Motoyama, M, Ohara, M, Suzuki, K, Igari, Y, Watanabe, K, Nakano, H, Oba, K: Urinary N-acetyl-beta-D-glucosaminidase levels are positively correlated with 2-hr plasma glucose levels during oral glucose tolerance testing in prediabetes. *J Clin Lab Anal,* 26**:** 473-480, 2012.
41. Tylicki, L, Manitius, J, Lysiak-Szydlowska, W, Rutkowski, B: Tubular injury: the first symptom of hypertensive kidney involvement? *Med Sci Monit,* 9**:** CR135-CR141, 2003.
42. Nangaku, M: Chronic hypoxia and tubulointerstitial injury: a final common pathway to end-stage renal failure. *J Am Soc Nephrol,* 17**:** 17-25, 2006.
43. Nauta, FL, Scheven, L, Meijer, E, van Oeveren, W, de Jong, PE, Bakker, SJL, Gansevoort, RT: Glomerular and Tubular Damage Markers in Individuals with Progressive Albuminuria. *Clin J Am Soc Nephrol,* 8**:** 1106-1114, 2013.

44. Brinkman, JW, de Zeeuw, D, Duker, JJ, Gansevoort, RT, Kema, IP, Hillege, HL, de Jong, PE, Bakker, SJ: Falsely low urinary albumin concentrations after prolonged frozen storage of urine samples. *Clin Chem,* 51**:** 2181-2183, 2005.
45. Trachtenberg, F, Barregard, L: Effect of storage time at −20°C on markers used for assessment of renal damage in children: albumin, γ-glutamyl transpeptidase, N-acetyl-β-d-glucosaminidase and α1-microglobulin. *Scand J Urol Nephrol,* 44**:** 331-336, 2010.
46. Jacobsen, BK, Eggen, AE, Mathiesen, EB, Wilsgaard, T, Njølstad, I: Cohort profile: The Tromsø Study. *Int J Epidemiol,* 41**:** 961-967, 2012.
47. Levey, AS, Stevens, LA, Schmid, CH, Zhang, YL, Castro, AF, III, Feldman, HI, Kusek, JW, Eggers, P, Van, LF, Greene, T, Coresh, J: A new equation to estimate glomerular filtration rate. *Ann Intern Med,* 150**:** 604-612, 2009.
48. The World Health Organization MONICA Project (monitoring trends and determinants in cardiovascular disease): a major international collaboration. WHO MONICA Project Principal Investigators. *J Clin Epidemiol,* 41**:** 105-114, 1988.
49. Luepker, RV, Apple, FS, Christenson, RH, Crow, RS, Fortmann, SP, Goff, D, Goldberg, RJ, Hand, MM, Jaffe, AS, Julian, DG, Levy, D, Manolio, T, Mendis, S, Mensah, G, Pajak, A, Prineas, RJ, Reddy, KS, Roger, VL, Rosamond, WD, Shahar, E, Sharrett, AR, Sorlie, P, Tunstall-Pedoe, H: Case definitions for acute coronary heart disease in epidemiology and clinical research studies: a statement from the AHA Council on Epidemiology and Prevention; AHA Statistics Committee; World Heart Federation Council on Epidemiology and Prevention; the European Society of Cardiology Working Group on Epidemiology and Prevention; Centers for Disease Control and Prevention; and the National Heart, Lung, and Blood Institute. *Circulation,* 108**:** 2543-2549, 2003.
50. Pencina, MJ, D'Agostino, RB, Sr., D'Agostino, RB, Jr., Vasan, RS: Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Statistics in medicine,* 27**:** 157-172, 2008.

Table 1. Baseline characteristics, the Tromsø Study 1994/95 (n=6834)

	Men (n=3369)		Women (n=3465)		P-value	Both genders (n=6834)	
Age, years	60	(±10)	61	(±10)	<0.001	60	(±10)
Urinary NAG, U/L	2.54	(1.90-3.48)	1.74	(1.25-2.42)	<0.001	2.12	(1.50-2.98)
Urinary NAG/creatinine ratio, U/gCr	1.93	(1.48-2.60)	1.95	(1.51-2.56)	0.43	1.94	(1.50-2.57)
Systolic blood pressure, mmHg	145	(±20)	145	(±24)	0.7	145	(±22)
Diastolic blood pressure, mmHg	85	(±12)	82	(±13)	<0.001	83	(±13)
Hypertension*, n (%)	2161	(64)	2044	(59)	<0.001	4205	(61)
Body mass index, kg/m^2	26.1	(±3.4)	26.0	(±4.5)	0.4	26.0	(±4.0)
Total cholesterol, mmol/L	6.51	(±1.21)	6.89	(±1.35)	<0.001	6.71	(±1.30)
HDL cholesterol, mmol/L	1.37	(±0.40)	1.65	(±0.44)	<0.001	1.51	(±0.44)
High sensitive CRP, mg/L	1.32	(0.69-2.80)	1.16	(0.57-2.46)	<0.001	1.25	(0.63-2.62)
Urinary albumin/creatinine ratio, mg/mmol	0.57	(0.36-1.16)	0.63	(0.42-1.05)	<0.001	0.61	(0.39-1.11)
Estimated GFR, ml/min/1.73 m^2	94	(±13)	92	(±14)	<0.001	93	(±14)
Antihypertensive drugs, n (%)	568	(17)	525	(15)	0.06	1093	(16)
Current smoker, n (%)	1166	(35)	1074	(31)	0.002	2240	(33)
Diabetes, n (%)**	158	(4.7)	149	(4.3)	0.4	307	(4.5)
Previous myocardial infarction, n (%)	305	(9.1)	102	(2.9)	<0.001	407	(6.0)
Previous ischemic stroke, n (%)	120	(3.6)	76	(2.2)	0.001	196	(2.9)

Values are given as mean (±SD), median (interquartile range) or number (%) as apporpriate. *Hypertension: systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or current use of blood pressure lowering medicaion. **Diabetes: self-reported diabetes mellitus or current use of antidiabetic medication or non-fasting serum glucose >10 mmol/L, or HbA1c ≥6.5%.

Table 2. Distribution of urinary NAG ratio and albumin/creatinine ratio (ACR) across age groups. The Tromsø Study 1994/95

	< 50 years (n=864)		50-59 years (n=1982)		60-69 years (n=2810)		≥ 70 years (n=1178)		P for linear trend
NAG ratio, U/g Cr	1.54	(1.21-2.02)	1.81	(1.42-2.33)	2.03	(1.58-2.71)	2.26	(1.79-3.12)	<0.001
ACR, mg/mmol	0.48	(0.35-0.73)	0.55	(0.37-0.90)	0.64	(0.41-1.20)	0.80	(0.48-1.82)	<0.001

Table 3. Cardiovascular risk factors by quartiles of urinary NAG ratio. The Tromsø Study 1994/95.

	I (≤1.49 U/g Cr)		II (1.50 - 1.93 U/g Cr)		III (1.94 - 2.56 U/g Cr)		IV (≥2.57 U/g Cr)		P value for linear trend
n	1708		1709		1709		1708		
Age, years	56	(±12)	60	(±9)	62	(±9)	64	(±8)	<0.001
Gender, % m/%f	51/49		48/52		47/53		50/50		0.48
Systolic blood pressure, mm Hg	140	(±21)	144	(±22)	146	(±22)	151	(±24)	<0.001
Diastolic blood pressure, mm Hg	81	(±12)	83	(±13)	84	(±13)	85	(±14)	<0.001
Hypertension*, n (%)	849	(50)	995	(58)	1124	(66)	1237	(73)	<0.001
Antihypertensive drugs, n (%)	171	(10)	249	(15)	339	(20)	483	(28)	<0.001
Body mass index, kg/m^2	25.7	(±3.6)	26.0	(±3.9)	26.0	(±4.0)	26.4	(±4.4)	<0.001
Total cholesterol, mmol/L	6.57	(±1.32)	6.76	(±1.27)	6.84	(±1.29)	6.82	(±1.28)	<0.001
HDL cholesterol, mmol/L	1.53	(±0.43)	1.54	(±0.42)	1.54	(±0.44)	1.52	(±0.45)	0.5
High sensitive CRP, mg/L	0.96	(0.50-1.95)	1.08	(0.58-2.27)	1.36	(0.70-2.69)	1.73	(0.81-3.78)	<0.001
Urinary albumin/creatinine ratio, mg/mmol	0.45	(0.32-0.71)	0.55	(0.38-0.87)	0.67	(0.44-1.20)	0.92	(0.53-2.11)	<0.001
Estimated GFR, ml/min/1.73 m^2	96	(±13)	93	(±12)	92	(±13)	90	(±15)	<0.001
Current smoker, n (%)	447	(26)	531	(31)	611	(36)	651	(38)	<0.001
Physically active, n (%)	475	(28)	366	(21)	336	(20)	272	(16)	<0.001
Diabetes, n (%)**	30	(1.8)	40	(2.3)	55	(3.2)	182	(10.7)	<0.001
Previous myocardial infarction, n (%)	58	(3.4)	87	(5.1)	98	(5.7)	164	(9.6)	<0.001
Previous ischemic stroke, n (%)	30	(1.8)	27	(1.6)	55	(3.2)	84	(4.9)	<0.001

Values are given as mean (±SD), median (interquartile range) or number (%) as apporpriate. *Hypertension: systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or current use of blood pressure lowering medicaion. **Diabetes: self-reported diabetes mellitus or current use of antidiabetic medication or non-fasting serum glucose >10 mmol/L, or HbA1c ≥6.5 %.

Table 4. Crude incidence rates for cardiovascular events and all-cause mortality according to quartiles of urinary NAG/creatinine ratio (NAG ratio). The Tromsø Study 1994/95.

A. First-ever myocardial infarction

NAG ratio quartiles	Number of Events	Crude Incidence Rate	95 %Confidence Interval	P Value*
I (≤1.49 U/g Cr)	155	6.73	5.67 - 7.79	Ref.
II (1.50 - 1.93 U/g Cr)	203	9.08	7.83 - 10.33	0.02
III (1.94 - 2.56 U/g Cr)	264	12.34	10.85 - 13.83	<0.001
IV (≥2.57 U/g Cr)	336	17.28	15.43 - 19.13	<0.001
Total	958	11.11	10.40 - 11.82	

B. First-ever ischemic stroke

NAG ratio quartiles	Number of Events	Crude Incidence Rate	95 %Confidence Interval	P Value*
I (≤1.49 U/g Cr)	110	4.73	3.85 - 5.61	Ref.
II (1.50 - 1.93 U/g Cr)	166	7.41	6.28 - 8.54	0.007
III (1.94 - 2.56 U/g Cr)	178	8.30	7.08 - 9.52	<0.001
IV (≥2.57 U/g Cr)	272	14.05	12.37 - 15.73	<0.001
Total	726	8.39	7.78 - 9.00	

C. All-cause mortality

NAG ratio quartiles	Number of Events	Crude Incidence Rate	95 %Confidence Interval	P Value*
I (≤1.49 U/g Cr)	306	10.92	9.70 - 12.14	Ref.
II (1.50 - 1.93 U/g Cr)	505	18.87	17.22 - 20.52	<0.001
III (1.94 - 2.56 U/g Cr)	647	25.57	23.60 - 27.54	<0.001
IV (≥2.57 U/g Cr)	900	39.25	36.69 - 41.81	<0.001
Total	2358	23.15	22.23 - 24.07	

*P value for comparison with the reference group.

Table 5. Hazard Ratios for cardiovascular events and all-cause mortality. The Tromsø Study 1994/95.

A. First-ever myocardial infarction

		Hazard Ratio	95% Confidence Interval	P-value
Multivariable model 1	**NAG ratio, per U/g Cr**	1.04	1.00 - 1.09	0.06
Multivariable model 2	**NAG ratio, quartile 1 (≤1.49 U/g Cr)**	1 (ref.)		
	NAG ratio, quartile 2 (1.50 - 1.93 U/g Cr)	1.09	0.88 - 1.35	0.4
	NAG ratio, quartile 3 (1.94 - 2.56 U/g Cr)	1.33	1.08 - 1.63	0.007
	NAG ratio, quartile 4 (≥2.57 U/g Cr)	1.43	1.16 - 1.76	<0.001

B. First-ever ischemic stroke

		Hazard Ratio	95% Confidence Interval	P-value
Multivariable model 1	**NAG ratio, per U/g Cr**	1.04	0.99 - 1.09	0.17
Multivariable model 2	**NAG ratio, quartile 1 (≤1.49 U/g Cr)**	1 (ref.)		
	NAG ratio, quartile 2 (1.50 - 1.93 U/g Cr)	1.20	0.94 - 1.53	0.15
	NAG ratio, quartile 3 (1.94 - 2.56 U/g Cr)	1.13	0.88 - 1.45	0.33
	NAG ratio, quartile 4 (≥2.57 U/g Cr)	1.41	1.10 - 1.80	0.006

C. All-cause mortality

		Hazard Ratio	95% Confidence Interval	P-value
Multivariable model 1	**NAG ratio, per U/g Cr**	1.1	1.07 - 1.12	<0.001
Multivariable model 2	**NAG ratio, quartile 1 (≤1.49 U/g Cr)**	1 (ref.)		
	NAG ratio, quartile 2 (1.50 - 1.93 U/g Cr)	1.24	1.08 - 1.44	0.003
	NAG ratio, quartile 3 (1.94 - 2.56 U/g Cr)	1.44	1.25 - 1.66	<0.001
	NAG ratio, quartile 4 (≥2.57 U/g Cr)	1.60	1.39 - 1.84	<0.001

All models were adjusted for gender, age, systolic blood pressure, current use of antihypertensive medication, body mass index, serum cholesterol, HDL cholesterol and high sensitive CRP, current smoking, diabetes, hard physical activity ≥1 hour per week, estimated GFR and log urinary albumin/creatinine ratio. In addition, models in A were adjusted for previous ischemic stroke, models in B were adjusted for previous myocardial infarction and in C for both.

Table 6. Multivariable adjusted Hazard Ratios for cardiovascular events and all-cause mortality by increased NAG ratio and/or increased albumin/creatinine ratio (ACR). The Tromsø Study 1994/95.

A. First-ever myocardial infarction

	Mean time (months) to event	Hazard Ratio	95% Confidence Interval	P-value
***Low NAG ratio and low ACR**	99.1 (50.1)	1 (ref.)		
Low NAG ratio and **high ACR	91.9 (53.4)	1.02	0.82 - 1.26	0.86
High NAG ratio and low ACR	101.1 (53.8)	1.21	0.98 - 1.48	0.07
High NAG ratio and high ACR	88.0 (50.1)	1.48	1.23 - 1.77	<0.001
	P for linear trend = 0.023			

B. First-ever ischemic stroke

	Mean time (months) to event	Hazard Ratio	95% Confidence Interval	P-value
Low NAG ratio and low ACR	110.5 (49.6)	1 (ref.)		
Low NAG ratio and high ACR	95.9 (52.5)	1.59	1.25 - 2.03	<0.001
High NAG ratio and low ACR	99.8 (56.2)	1.21	0.93 - 1.56	0.15
High NAG ratio and high ACR	91.3 (50.8)	1.79	1.44 - 2.22	<0.001
	P for linear trend = 0.002			

C. All-cause mortality

	Mean time (months) to event	Hazard Ratio	95% Confidence Interval	P-value
Low NAG ratio and low ACR	135.0 (56.6)	1 (ref.)		
Low NAG ratio and high ACR	133.6 (55.9)	1.31	1.14 - 1.51	<0.001
High NAG ratio and low ACR	125.4 (57.2)	1.36	1.19 - 1.56	<0.001
High NAG ratio and high ACR	114.4 (59.3)	1.80	1.60 - 2.04	<0.001
	P for linear trend <0.001			

*Low: below median of the variable. **High: median of the variable and above. All models were adjusted for gender, age, systolic blood pressure, current use of antihypertensive medication, body mass index, serum cholesterol, HDL cholesterol and high sensitive CRP, current smoking, diabetes, hard physical activity ≥1 hour per week, and estimated GFR. In addition, models in A were adjusted for previous ischemic stroke, models in B were adjusted for previous myocardial infarction and in C for both.

Table 7. Area under the ROC curve (AUC) and Net Reclassification Improvement for the prediction of three endpoints, using different prediction models. The Tromsø Study 1994/95.

	Myocardial Infarction		Ischemic Stroke		All-cause Mortality	
AUC Model 1c (95% Confidence Interval)	0.706	(0.690 - 0.722)	0.726	(0.708 - 0.744)	0.818	(0.807 - 0.828)
AUC Model 2c (95% Confidence Interval)	0.707	(0.691 - 0.724)	0.732	(0.714 - 0.750)*	0.821	(0.811 - 0.832)*
AUC Model 3c (95% Confidence Interval)	0.710	(0.694 - 0.726)*	0.733	(0.715 - 0.750)*	0.824	(0.814 - 0.835)*†
NRI_{yes} Model 1c vs. Model 2c	0.006	P=0.4	0.023	P=0.10	0.006	P=0.35
NRI_{no} Model 1c vs. Model 2c	0.001	P=0.7	0.003	P=0.6	0.003	P=0.55
$NRI_{overall}$ Model 1c vs. Model 2c	0.007	P=0.4	0.025	P=0.08	0.009	P=0.27
NRI_{yes} Model 2c vs. Model 3c	0.001	P=0.9	0.023	P=0.10	0.009	P=0.21
NRI_{no} Model 2c vs. Model 3c	0	P=1	0.003	P=0.6	0.004	P=0.40
$NRI_{overall}$ Model 2c vs. Model 3c	0.001	P=0.9	0.025	P=0.08	0.013	P=0.13

Model 1c: gender, age, systolic blood pressure, current use of antihypertensive medication, body mass index, serum cholesterol, HDL cholesterol and high sensitive CRP, current smoking, diabetes, hard physical activity ≥1 hour per week and previous cardiovascular disease (ischemic stroke for the prediction of myocardial infarction, myocardial infarction for the prediction of stroke and both for the prediction of all-cause mortality).

Model 2c: Model 1c + eGFR (in quartiles) and urinary ACR (in quartiles)

Model 3c: Model 2c + NAG ratio (in quartiles)

NRI_{yes}: Net Reclassification Improvement for the group who experienced the event. NRI_{no}: Net Reclassification Improvement for the group who did not experienced the event. $NRI_{overall}$: Overall Net Reclassification Improvement.

*P<0.05 for comparison with Model 1c. †P<0.05 for comparison with Model 2c.