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Association of urinary KIM-1, L-FABP, NAG and NGAL with incident end-stage renal disease and mortality in American Indians with type 2 diabetes mellitus

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Duality of interest

JVB appears as co-inventor on KIM-1 patents, which have been licensed by Partners Healthcare to a number of companies. He has received royalty income from Partners Healthcare. KDL had reagents donated for previous biomarker studies by Abbott and CMIC. All other authors declare that there is no duality of interest associated with their contribution to this manuscript..

Contribution statement

RGN, CYH, KDL, GDF, EJW, JVB and SSW designed the study with input into the study protocol from all authors. TEM, VS and JVB performed the biomarker assays. GDF, EJW, RGN, RLH, XZ and DX performed the statistical analyses. GDF, EJW, RGN and KDL drafted the manuscript and all authors assisted with revising it critically for important intellectual content. All authors contributed to the interpretation of the data and approved the final version. RGN is the guarantor of this work.

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Abstract

Aims/hypothesis—Kidney injury molecule 1 (KIM-1), liver fatty acid-binding protein (L-FABP), *N*-acetyl-β-D-glucosaminidase (NAG) and neutrophil gelatinase-associated lipocalin (NGAL) are urinary biomarkers of renal tubular injury. We examined their association with incident end-stage renal disease (ESRD) and all-cause mortality in American Indians with type 2 diabetes.

Methods—Biomarker concentrations were measured in baseline urine samples in 260 Pima Indians who were followed for a median of 14 years. HRs were reported per SD of creatinine (Cr)-normalised log-transformed KIM-1, NAG and NGAL, and for three categories of L-FABP.

Results—During follow-up, 74 participants developed ESRD and 101 died. Median concentrations of KIM-1/Cr, NAG/Cr and NGAL/Cr and the proportion of detectable L-FABP were highest in those with macroalbuminuria ($p < 0.001$ for KIM-1/Cr, NAG/Cr and L-FABP; $p = 0.006$ for NGAL/Cr). After multivariable adjustment, NGAL/Cr was positively associated with ESRD (HR 1.59, 95% CI 1.20, 2.11) and mortality (HR 1.39, 95% CI 1.06, 1.82); L-FABP/Cr was inversely associated with ESRD (HR [for highest vs lowest tertile] 0.40, 95% CI 0.19, 0.83). Addition of NGAL/Cr to models that included albuminuria and glomerular filtration rate increased the c-statistic for predicting ESRD from 0.828 to 0.833 ($p = 0.001$) and for death from 0.710 to 0.722 ($p = 0.018$). Addition of L-FABP/Cr increased the c-statistic for ESRD from 0.828 to 0.832 ($p = 0.042$).

Conclusions/interpretation—In Pima Indians with type 2 diabetes, urinary concentrations of NGAL and L-FABP are associated with important health outcomes, but they are unlikely to add to risk prediction with standard markers in a clinically meaningful way given the small increase in the c-statistic.

Keywords

Biomarkers; End-stage renal disease; Mortality; Type 2 diabetes

Introduction

Type 2 diabetes is the leading cause of chronic kidney disease (CKD) and kidney failure in the USA, accounting for 44% of new cases of end-stage renal disease (ESRD) in 2011 [1]. Albuminuria is the best currently available risk marker for progressive CKD attributable to diabetes, but it has a number of drawbacks. First, it may return to normal spontaneously or in response to therapy in many persons with diabetes [2, 3]. Second, the estimate of a patient's risk of CKD progression depends predominantly on the results of the most recent test [4, 5], suggesting that past measures of albuminuria have only modest relevance to the

risk of future kidney disease. Finally, the absence of increased albuminuria does not preclude the presence of diabetic kidney disease [6, 7]. Therefore, investigators are searching for new biomarkers of diabetic kidney disease that provide additional prognostic information beyond that provided by albuminuria.

Potential biomarkers of kidney disease in type 2 diabetes include molecules predominantly expressed by renal tubular cells, including kidney injury molecule 1 (KIM-1), liver fatty acid-binding protein (L-FABP), N-acetyl-β-D-glucosaminidase (NAG) and neutrophil gelatinase-associated lipocalin (NGAL). Each of these biomarkers has been evaluated previously in relation to diabetic kidney disease, often with conflicting results [8–23]. This variation may be due to differences in study design, inclusion of persons without diabetes or with type 1 diabetes, use of surrogate or composite outcomes or incomplete covariate adjustment in risk models. Here we explore the association of these biomarkers with outcomes of ESRD and all-cause mortality in a cohort of American Indians with type 2 diabetes and normal measured glomerular filtration rate (GFR). Half of the participants had elevated urinary albumin excretion at baseline.

Methods

The CKD Biomarkers Consortium was established in 2008 by the National Institute of Diabetes and Digestive and Kidney Diseases to advance the field of CKD biomarker discovery and validation. The Phoenix Epidemiology and Clinical Research Branch is part of this consortium. Urine samples and phenotypic information acquired from studies conducted at this facility were used in the present analysis. These studies were approved by the review board of the National Institute of Diabetes and Digestive and Kidney Diseases. Each participant gave informed consent.

Study population

Between 1965 and 2007, Pima Indians from the Gila River Indian Community participated in a longitudinal study of diabetes and its complications. Each member of this community who was at least 5 years of age was invited to undergo a research examination approximately every 2 years. Diabetes was diagnosed by a 2 h post-load plasma glucose concentration ≥ 11.1 mmol/l (200 mg/dl) at these biennial examinations, or when the diagnosis was documented in the medical record. For the present study, we selected participants from this longitudinal population-based study who were ≥ 18 years old, had type 2 diabetes and also participated in one of two longitudinal studies of kidney function that included measurements of GFR by the urinary clearance of iothalamate [24, 25]. The date of diabetes diagnosis for each participant was ascertained from the longitudinal population-based study; all other variables used in this analysis were measured at the first kidney function study at which the participant met eligibility requirements for the present analysis. This study was considered the participant's baseline examination, and bio-banked urine collected at this examination was used for measurement of biomarker concentrations. Baseline examinations were conducted between April 1990 and December 2003.

Participants were followed until ESRD, death or 31 December 2013. ESRD was defined by the initiation of renal replacement therapy or death from diabetic kidney disease if the

participant refused dialysis. Surveillance for ESRD and death were conducted independently of the research examinations, and causes of death were determined by systematic review of death certificates and all available medical records. The vital status of all study participants was confirmed until 31 December 2013.

Measurements

BMI was defined as weight divided by the square of height (kg/m^2). Blood pressure was measured twice with the participant resting in the seated position and the results were averaged. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or treatment with antihypertensive medicine. HbA_{1c} was measured by HPLC. GFR was measured in the morning, after an overnight fast, by the urinary clearance of non-radioactive iothalamate averaged over four carefully timed 20 min urine collections and bracketed by the collection of blood samples. These measurements were made after a water load and a 60 min equilibration period. An HPLC system with a sensitive ultraviolet light detector was used to assay iothalamate at 236 nm (Instrumentation Shimadzu #6A; Shimadzu, Kyoto, Japan) [26]. Urinary albumin was measured at the time of collection by nephelometric immunoassay and urinary creatinine (Cr) by a modified Jaffé reaction. Urinary albumin excretion was estimated from a single spot urine collection by computing the albumin-to-creatinine ratio (Alb/Cr) in units of mg/g (converted to SI units of mg/mmol). Normoalbuminuria was defined as Alb/Cr < 3.5 mg/mmol (< 30 mg/g), microalbuminuria as 3.5 – 30 mg/mmol (30–299 mg/g) and macroalbuminuria as ≥ 30 mg/mmol (≥ 300 mg/g). Remaining serum and urine samples were placed in long-term storage at −80°C.

Urinary albumin, Cr, KIM-1, L-FABP, NAG and NGAL were measured in 2012 in stored urine samples that had undergone a maximum of two prior freeze–thaw cycles. Urine albumin was measured by an immunoturbidimetric assay and urine Cr by a kinetic colorimetric assay on an automated analyser (Roche, Basel, Switzerland). KIM-1 was measured by a microbead-based sandwich ELISA on a Bioplex-200 platform (Bio-Rad, Hercules, CA, USA), L-FABP by a two-step sandwich ELISA assay (CMIC, Tokyo, Japan), NAG by an enzymatic assay (Roche, Indianapolis, IN, USA) and NGAL by a non-competitive sandwich assay with chemiluminescent signal detection on an ARCHITECT platform (Abbott Diagnostics, Abbott Park, IL, USA). Reproducibility of the biomarker assays was assessed by intra-class correlation of measurements from 50 duplicate samples blinded to the performance laboratories. Intra-class correlation for non-normalised KIM-1 was 0.98, for L-FABP was 0.95, for NAG was 0.95 and for NGAL was 0.99, reflecting excellent agreement.

Storage time of baseline urine samples prior to performance of biomarker assays was bimodal because specimens were derived from two different study cohorts that underwent identical kidney function testing [24, 25]. Of the 260 participants included in this study, 141 were from the first study cohort and 119 were from the second study cohort. Median storage time for the first cohort was 21.3 (interquartile range [IQR] 20.7–21.7) years and for the second cohort was 12.0 (IQR 11.7–12.3) years. Biomarker concentrations were normalised to urine Cr concentration to adjust for variability in urine concentration and to account for

any desiccation that occurred during long-term storage. Concentrations of albumin in three samples and NAG in two samples were undetectable and reported as zero by the performance laboratories. To analyse these samples as continuous variables, a value of 1/10 of the lower limit of detection was arbitrarily assigned for analysis. Because 107 L-FABP samples were below the detection limit of the assay in the performance laboratory, analyses involving this analyte were categorised: those below the limit of detection (LOD) were included in the lowest category; those below the median of detectable values in the middle category and those at or above the median in the highest category.

Statistical methods

Clinical features at baseline were described using means and SDs for normally distributed variables and medians and IQRs for those not normally distributed. Median normalised biomarker concentrations between men and women were compared using the Wilcoxon two-sample test. Median normalised biomarker levels by albuminuria group were compared by the Kruskal–Wallis test and detectable vs undetectable L-FABP levels by albuminuria group were compared by the Cochran–Armitage trend test. Spearman's correlations of normalised and log-transformed urinary biomarker concentrations with other CKD risk factors were calculated along with their *p* values. Correlations between biomarkers were partialled to remove the potentially spurious effect of urine Cr caused by normalising each biomarker for this variable. A low value of 0.01 was assigned for values of L-FABP below the LOD for these calculations. Kaplan–Meier survival curves for the outcomes of ESRD and mortality were plotted by tertiles of urinary concentrations of Alb/Cr, KIM-1/Cr, NAG/Cr and NGAL/Cr and by the three categories of L-FABP/Cr defined above; logrank statistics were calculated to examine differences in the probability of reaching the specified health outcomes by these biomarker categories. The associations of individual urinary biomarkers were further examined using Cox proportional hazards regression with the urinary biomarkers divided into tertiles (or the three categories of L-FABP/Cr) or logarithmically transformed. For the logarithmically transformed analyses, the HR was expressed for a 1-SD increment in the distribution of the natural logarithm of the biomarker. Four Cox models were described for each urinary biomarker: (1) univariate; (2) adjusted for baseline age, sex, diabetes duration, hypertension, HbA_{1c} and study cohort; (3) adjusted for the covariates in model 2 plus GFR and (4) adjusted for the covariates in model 3 plus the natural logarithm of Alb/Cr.

Three sets of sensitivity analyses were performed. The first set of analyses substituted in the Cox models the Alb/Cr values measured at the time of sample collection for those measured by the CKD Biomarkers Consortium after long-term storage. The same assay methods were used for both sets of measurements. The second set of analyses removed from consideration in the Cox models the five individuals with arbitrarily assigned data values for undetectable Alb/Cr and NAG/Cr values. The third analysis removed from consideration in the Cox models the eight participants who were taking angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers at the time the biomarker samples were collected.

Generalised c-statistics were calculated for each model accounting for variable follow-up times [27]. Comparisons between nested models that included or excluded the analyte of

interest were assessed by likelihood ratio tests [28, 29]. The magnitude of improvement in discrimination of the 13 year risk for the outcomes of interest with the addition of each biomarker was assessed by relative integrated discrimination improvement (IDI) [30, 31]. The 13 year risk was selected, since this was approximately the median follow-up time for each health outcome of interest. The 95% CIs for the changes in the c-statistic and the relative IDI were computed based on 10,000 bootstrap samples. Analyses were performed using SAS software version 9.3 (SAS Institute, Cary, NC, USA).

Results

Clinical characteristics of the 260 Pima Indians and the median normalised biomarker concentrations at baseline are shown according to categories of albuminuria in Table 1. The mean age of the participants was 42.5 (range 18.7–65.1) years and the mean duration of diabetes was 11.4 (0.0–39.8) years. The mean GFR at baseline was 149 (26–265) ml/min. Median concentrations of KIM-1/Cr, NAG/Cr and NGAL/Cr differed significantly from one another by albuminuria category, with the highest concentrations in those with macroalbuminuria ($p < 0.001$ for KIM-1/Cr and NAG/Cr; $p = 0.006$ for NGAL/Cr) (Fig. 1). The proportion of detectable L-FABP was also highest in those with macroalbuminuria ($p < 0.001$). The concentration of NGAL/Cr was significantly higher ($p < 0.001$) in women (median [IQR] 9.4 [5.3–18.3] ng/mmol (or 83.2 [47.2–162.1] ng/mg) than in men 1.8 [1.2–3.2] ng/mmol (or 16.3 [10.9–28.3] ng/mg). No sex differences were found for the other biomarkers, including the urinary Alb/Cr. Spearman's correlations partialled for urinary creatinine are shown in Table 2 for the relationships between the log-transformed urinary biomarkers and other CKD risk factors. Correlations were generally modest, with the strongest correlation observed between Alb/Cr and L-FABP/Cr ($r = 0.601$, $p < 0.001$).

Participants were followed for a median (IQR) of 13.5 (9.6–15.4) years for ESRD and 13.8 (12.6–20.8) years for mortality. During follow-up, 74 participants developed ESRD and 101 died. Forty-eight participants who developed ESRD subsequently died. Kaplan–Meier survival plots for ESRD and all-cause mortality by tertiles of Alb/Cr, KIM-1/Cr, NAG/Cr and NGAL/Cr are shown in Fig. 2 and for undetected L-FABP vs detected L-FABP/Cr below the median vs detected L-FABP/Cr at or above the median in Fig. 3. Significant differences in the probability of developing ESRD were found by these categories of Alb/Cr ($p < 0.001$), L-FABP/Cr ($p < 0.001$), NAG/Cr ($p = 0.003$) and NGAL/Cr ($p < 0.001$) and with all-cause mortality for categories of Alb/Cr ($p < 0.001$), KIM-1/Cr ($p = 0.003$), L-FABP/Cr ($p = 0.010$) and NAG/Cr ($p = 0.005$).

In Cox proportional hazards models that examined HRs for ESRD by biomarker concentrations normalised to urinary creatinine, NGAL/Cr was positively associated with ESRD in both the categorical and continuous analyses after adjusting for CKD covariates, including GFR and Alb/Cr, whereas L-FABP was inversely associated (Table 3). In the fully-adjusted models, the risk of ESRD relative to the lowest tertile of NGAL/Cr was significantly higher for both the middle tertile (HR 3.76, 95% CI 1.67, 8.47) and the highest tertile (HR 6.88, 95% CI 2.91, 16.31). Conversely, the risk of ESRD was significantly lower in the highest tertile of L-FABP/Cr relative to the lowest tertile (HR 0.40, 95% CI 0.19, 0.83). In the continuous analysis, each 1 SD increase in log-transformed NGAL/Cr was

associated with an increased risk of ESRD (HR 1.59, 95% CI 1.20, 2.11). In models that examined associations with all-cause mortality, NGAL/Cr was positively associated with all-cause mortality in the continuous but not the categorical analysis (Table 4). The addition of NGAL/Cr as a continuous variable to the Cox regression models that included GFR and Alb/Cr increased the c-statistic for predicting ESRD from 0.828 to 0.833 ($p = 0.001$) and for predicting death from 0.710 to 0.722 ($p = 0.018$). The addition of L-FABP/Cr increased the c-statistic for ESRD from 0.828 to 0.832 ($p = 0.042$). The addition of these biomarkers also improved relative integrated discrimination for predicting ESRD by 2.9% for NGAL/Cr and 3.3% for L-FABP and for predicting death by 7.0% after 13 years of follow-up (Table 5). The conclusions of the study were unchanged in the sensitivity analyses or when BMI and serum cholesterol concentration were added to the Cox models (data not shown).

Discussion

Although numerous studies have examined KIM-1, L-FABP, NAG and NGAL as biomarkers of renal tubular injury in various settings, few studies have conducted direct comparisons of biomarker performance. We found that NGAL had the strongest associations with ESRD and all-cause mortality of the four biomarkers we evaluated. NGAL enhanced the discrimination of the survival models for each health outcome beyond that achievable by the clinically recognised risk factors, including Alb/Cr and GFR, when examined using the c-statistic. These findings suggest that NGAL adds information to the risk assessment for ESRD and mortality in Pima Indians with type 2 diabetes but the magnitude of the improvement in risk assessment is modest. Urinary NGAL concentration was significantly higher in women than men with type 2 diabetes, which is consistent with the sex difference reported in individuals with type 1 diabetes and with our observations made in a more general CKD population [18, 32].

Of note, L-FABP was below the LOD (2.4 ng/ml) in a large proportion of the Pima Indian cohort. Detectable concentrations of this analyte at or above the median were associated with a nearly threefold higher risk of ESRD and a twofold higher risk of death relative to undetectable concentrations in univariate analysis, but with a 60% lower risk of ESRD and a 13% lower risk of death (although the latter was not statistically significant) after adjustment for Alb/Cr and GFR. The strong correlation between L-FABP and Alb/Cr may be responsible for these findings. The correlation is likely attributable to the binding of NEFAs to albumin, which in turn stimulates the expression of L-FABP [33, 34]. Nevertheless, although higher concentrations of L-FABP have been associated previously with more advanced diabetic kidney disease, including ESRD [9, 19, 22, 23], the Translation Research Investigating Biomarker Endpoints for Acute Kidney Injury (TRIBE-AKI) study recently noted that urinary L-FABP was inversely associated with mortality among participants who underwent cardiac surgery without developing acute kidney injury after adjustment for estimated GFR and albuminuria, whereas none of the other biomarkers they evaluated, including urinary NGAL, KIM-1 and IL-18, had this inverse relationship [35].

The tubular biomarkers explored in this study associate strongly with acute kidney injury and therefore may not capture as strongly the relevant pathological processes operating in early diabetic kidney disease. Biomarkers that more closely reflect the pathological

processes occurring in the tubules early in type 2 diabetes may associate more strongly with progression of this disease, thereby complementing or adding additional predictive information to the biomarkers measured in this study. Structural changes in the glomerular compartment also occur early in diabetic kidney disease, so biomarkers reflecting glomerular injury may also predict health outcomes in type 2 diabetes. NGAL, unlike the other three tubular biomarkers we evaluated, is filtered by the glomerulus [36] and is a marker of inflammation [37]. These two factors might explain why NGAL was more strongly associated with progressive kidney disease in the current study as well as in some previous studies [8, 12, 14, 18].

Tubular markers may associate more strongly with progression of glomerular kidney diseases that are characterised by considerable proteinuria, as the proteinuria may promote tubular damage, thereby accelerating the progression of CKD [38]. In the present study, the median Alb/Cr was only 3.2 mg/mmol (28.3 mg/g), indicating that most participants did not have high levels of proteinuria at the baseline examination. Tubular markers may also associate with progression of glomerular kidney diseases when acute tubular necrosis occurs simultaneously with diabetic glomerular disease. A recent review of clinically indicated kidney biopsies in 620 patients with diabetes reported a significant association between the presence of diabetic glomerulosclerosis and acute tubular necrosis [39].

Urinary NGAL, but not NAG, KIM-1 or L-FABP, was associated with all-cause mortality in the present study. The number of deaths in our cohort was too small to assess the role of this biomarker in cause-specific mortality. In a community-based cohort of Swedish men [40], urinary NGAL was associated with cardiovascular and all-cause mortality independent of cardiovascular risk factors and GFR but the association disappeared when urinary albumin was included in the analysis. Serum NAG was previously associated with cardiovascular and all-cause mortality in a cohort of 1,070 apparently healthy Japanese individuals [41] and urinary NAG was associated with all-cause mortality among persons with heart failure [42].

Framingham investigators reported an association between urinary KIM-1 and mortality in a large cohort of apparently health individuals [20]. Urinary KIM-1 was also associated with mortality among individuals undergoing cardiac surgery in the TRIBE-AKI study [35] and in elderly individuals in the Health, Aging and Body Composition (Health ABC) study [43]. Finally, urinary L-FABP was associated with all-cause mortality in the Danish type 1 diabetes cohort in which it was also associated with progression to diabetic nephropathy, as described above [9].

Potential limitations of this study include prolonged storage time between sample collection and biomarker measurement. Although these samples were all stored at −80°C, there are limited data on the stability of these analytes at this temperature, and the stability of measurements may vary depending on the specific assay used for each analyte. Moreover, little is known about the impact of repeated freeze–thaw cycles on the stability of these analytes. The stability of KIM-1 and NGAL measurements was not significantly affected in urine samples subjected to several freeze–thaw cycles and stored for two years at −80°C [44], but this observation cannot necessarily be extrapolated to samples stored for many more years.

ACE inhibitors [45] and angiotensin receptor blockers [46] may affect biomarker concentrations in the urine by reducing the excretion of the markers or by causing acute kidney injury. Only 8 (3.1%) of the 260 participants were taking ACE inhibitors or angiotensin receptor blockers at the time of sample collection; the majority of those with elevated urinary albumin excretion were enrolled before use of these medicines became widespread in the community. Exclusion of participants who were taking these medicines from the analysis did not change our conclusions. Nevertheless, use of these medicines is now the standard of care, so the results of this study may not readily translate to current practice.

Strengths of this study include the longitudinal study design, the detailed characterisation of the study population and the large number of health outcomes. GFR was measured by the urinary clearance of iothalamate and health outcomes were important clinical endpoints that were systematically ascertained rather than surrogate outcomes.

In conclusion, urinary concentrations of the renal tubular markers NGAL and L-FABP were associated with important health outcomes in Pima Indians with type 2 diabetes, even after adjustment for baseline albuminuria and GFR. These biomarkers enhanced the identification of persons most likely to progress to ESRD (NGAL and L-FABP) or to premature death (NGAL) after considering traditional risk factors, but they are unlikely to add to risk prediction in a clinically meaningful way given the small increase in the c-statistic. Further studies are needed to determine whether these markers are associated with these adverse outcomes in other populations with type 2 diabetes, as well as in more general CKD populations.

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Abbreviations

ACE	Angiotensin-converting enzyme
Alb/Cr	Urinary albumin-to-creatinine ratio
CKD	Chronic kidney disease
Cr	Creatinine
ESRD	End-stage renal disease

GFR	Glomerular filtration rate
IDI	Integrated discrimination improvement
IQR	Interquartile range
KIM-1	Kidney injury molecule 1
L-FABP	Liver fatty acid-binding protein
LOD	Limit of detection
NAG	N-acetyl-β-D-glucosaminidase
NGAL	Neutrophil gelatinase-associated lipocalin
TRIBE-AKI	Translation Research Investigating Biomarker Endpoints for Acute Kidney Injury

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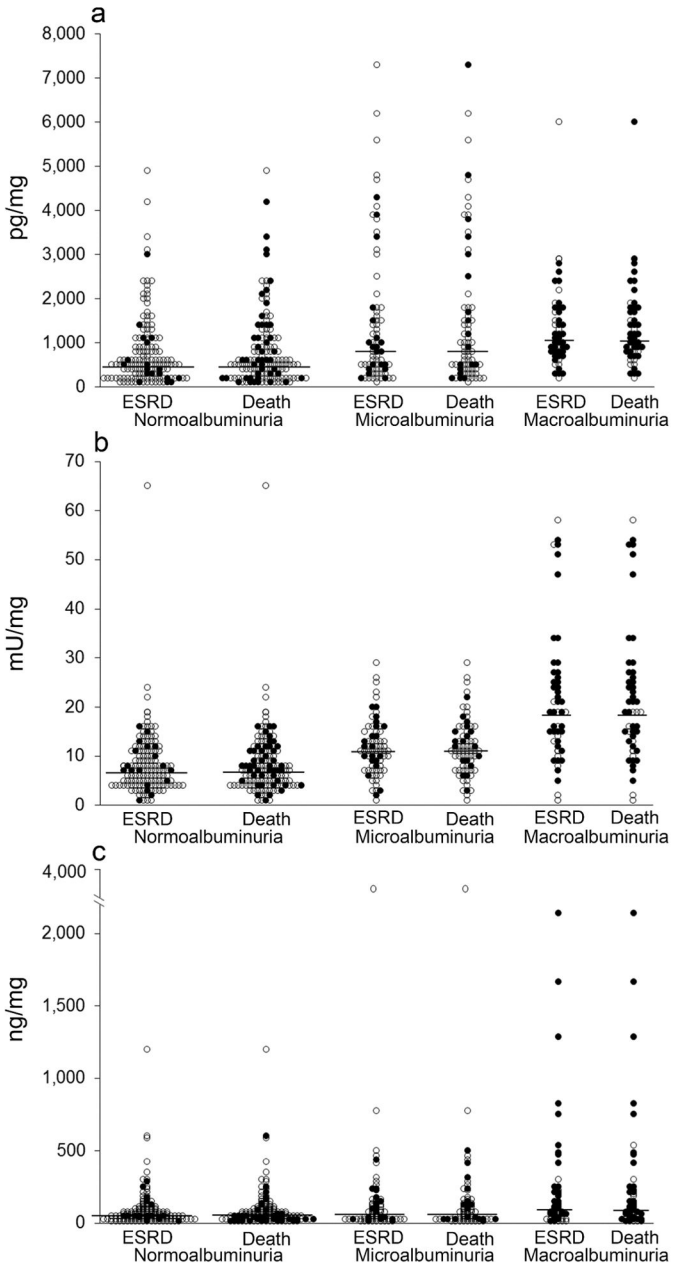

Fig. 1.

Distributions of urinary biomarker concentrations by level of Alb/Cr at baseline. Medians are represented by lines. Outcomes of ESRD and all-cause mortality are shown by black circles. (**a**) KIM-1/Cr; (**b**) NAG/Cr; (**c**) NGAL/Cr

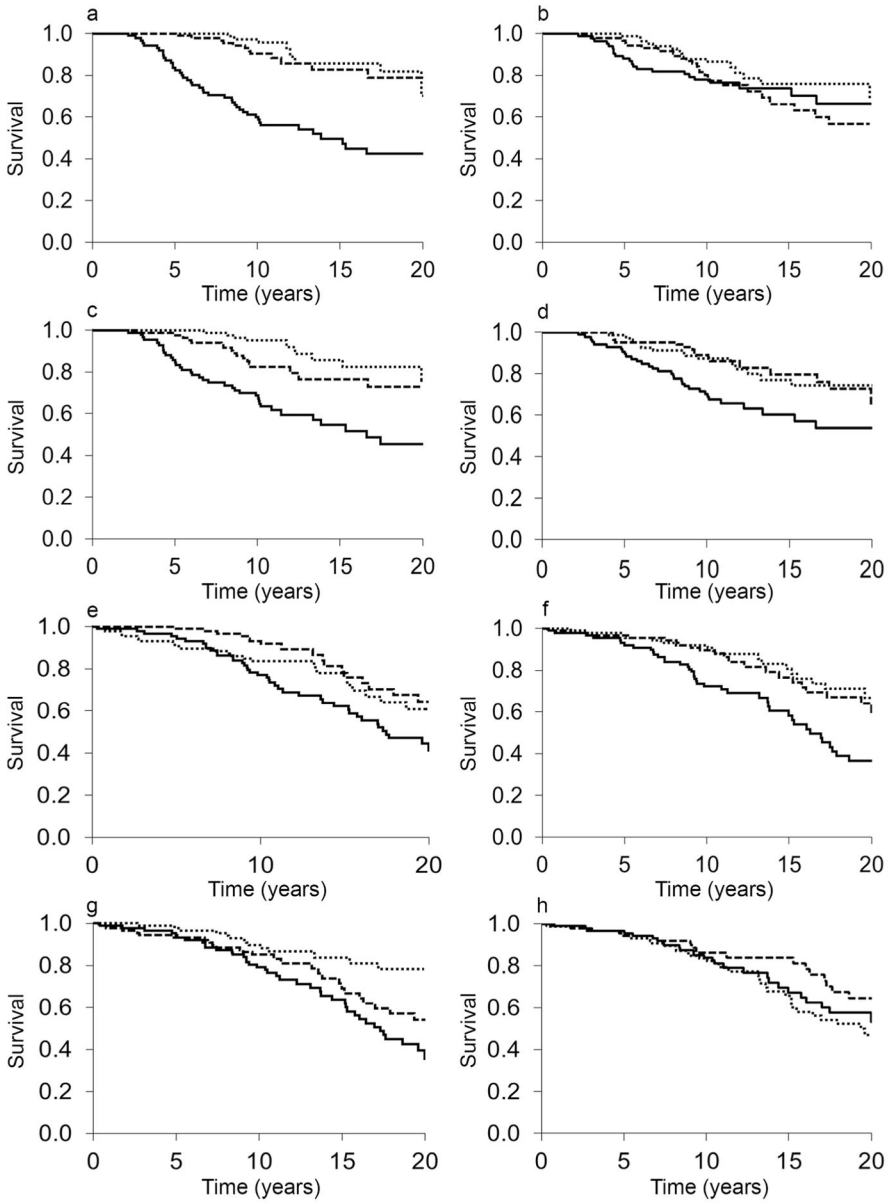

Fig. 2.

Kaplan–Meier survival curves for ESRD (**a–d**) and all-cause mortality (**e–h**) by tertiles (dotted line, lowest tertile; dashed line, middle tertile; solid line, highest tertile): Alb/Cr, $p<0.001$ (**a**, **e**); KIM-1/Cr, $p=0.362$ (**b**) and $p=0.003$ (**f**); NAG/Cr, $p=0.003$ (**c**) and $p=0.005$ (**g**); NGAL/Cr, $p<0.001$ (**d**) and $p=0.051$ (**h**). *p* values were calculated from the log-rank statistic

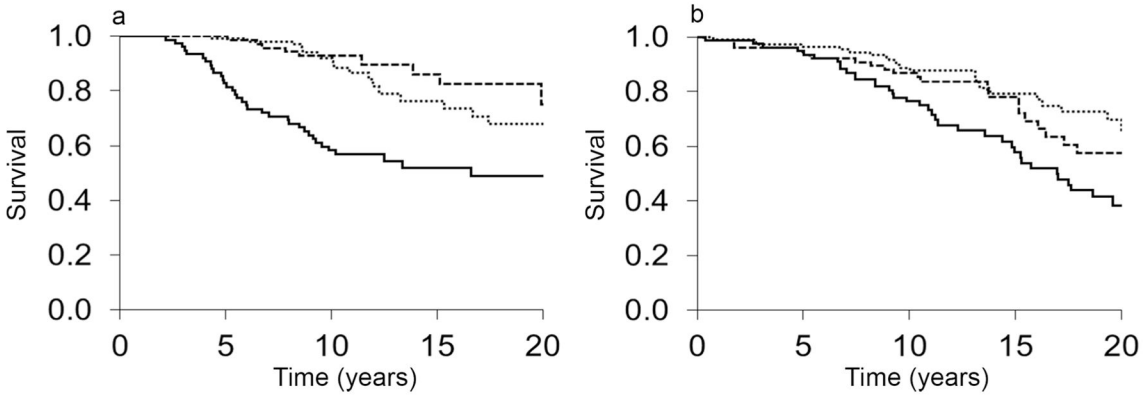

Fig. 3.

Kaplan–Meier survival curves for ESRD (**a**) and all-cause mortality (**b**) by undetected (dotted line), below detected median (dashed line) and detected median or above (solid line) of L-FABP/Cr. ESRD $p<0.001$ (**a**) and all-cause mortality $p=0.010$ (**b**). *p* values were calculated from the log-rank statistic

Table 1

Baseline characteristics of the 260 Pima Indians with type 2 diabetes

Characteristic	Normoalbuminuria	Microalbuminuria	Macroalbuminuria	Total
n	138	72	50	260
Sex, male	37 (26.8)	25 (34.7)	20 (40.0)	82 (31.5)
Age, years	40.8 ± 10.9	42.2 ± 10.2	47.4 ± 8.2	42.5 ± 10.5
Diabetes duration, years	9.0 ± 6.0	12.2 ± 6.3	16.6 ± 5.6	11.4 ± 6.6
BMI, kg/m^2	35.9 ± 8.5	35.9 ± 8.8	32.5 ± 7.0	35.3 ± 8.4
Blood pressure, mmHg				
Systolic	118 ± 14	121 ± 13	129 ± 16	121 ± 15
Diastolic	74 ± 8	76 ± 9	80 ± 10	76 ± 9
HbA_{1c}, %	8.9 ± 2.3	9.7 ± 2.3	10.4 ± 2.1	9.4 ± 2.4
HbA_{1c}, mmol/mol	73.8 ± 19.1	82.5 ±19.1	90.2 ± 17.4	79.2 ± 19.9
GFR, ml/min	154 ± 41	157 ± 43	122 ± 48	149 ± 45
RAS agent, *n*	4 (2.9)	2 (2.8)	2 (4.0)	8 (3.1)
Urinary Alb/Cr, mg/mmol	1.0 (0.6 – 1.9)	9.2 (5.1 – 17.3)	127.1 (55.8 – 194.5)	3.2 (0.9 – 19.5)
Urinary KIM-1/Cr, pg/mmol	51.9 (22.0–130.1)	91.5 (39.2–200)	118 (85.6–195)	76.2 (35.6–155)
Detectable urinary L-FABP	62 (44.9)	44 (61.1)	479 (4.0)	153 (58.8)
Urinary NAG/Cr, mU/mmol	0.768 (0.452–1.22)	1.22 (0.802–1.62)	2.07 (1.37–2.86)	1.08 (0.633–1.62)
Urinary NGAL/Cr, ng/mmol	5.39 (2.35–10.0)	6.92 (2.26–15.2)	9.81(3.22–25.0)	6.25 (2.46–13.71)

Data are means ± SD, median (25th–75th percentiles) or *n* (%)

Three values for Alb/Cr and two for NAG/Cr were undetectable

Table 2

Spearman's correlations between normalised and log-transformed urinary biomarkers and clinical characteristics in 260 Pima Indians with type 2 diabetes

Urinary biomarker	Age	Duration of diabetes	SBP	HbA_{1c}	GFR	Alb/Cr	KIM-1/Cr	NAG/Cr	NGAL/Cr	L-FABP/Cr
Alb/Cr	0.135 (0.032)	0.406 (<0.001)	0.223 (<0.001)	0.269 (<0.001)	−0.125 (0.001)	1.0				
KIM-1/Cr	0.187 (0.003)	0.160 (0.010)	0.183 (0.003)	0.022 (0.726)	−0.100 (0.112)	0.329 (<0.001)	1.0			
NAG/Cr	0.138 (0.028)	0.310 (<0.001)	0.153 (0.015)	0.403 (<0.001)	0.502 (<0.001)	−0.073 (0.245)	0.317 (<0.001)	1.0		
NGAL/Cr	−0.209 (0.001)	0.026 (0.676)	−0.091 (0.147)	0.277 (<0.001)	0.035 (0.573)	0.153 (0.015)	0.117 (0.061)	0.360 (<0.001)	1.0	
L-FABP/Cr	0.244 (<0.001)	0.310 (<0.001)	0.212 (0.001)	0.073 (0.243)	−0.371 (<0.001)	0.601 (<0.001)	0.216 (0.001)	0.378 (<0.001)	0.068 (0.276)	1.0

Results are shown as r (*p*)

Three values for Alb/Cr and two for NAG/Cr were undetectable. A low value of 0.01 was assigned for values of L-FABP below the LOD for these calculations. Correlations were partialled for urinary creatinine concentration SBP, systolic blood pressure

Table 3

HRs and 95% CIs for ESRD by normalised urinary biomarker

Urinary biomarker	ESRD (*n*=63/260)			
	Model 1 (univariate)	Model 2 (covariates[a])	Model 3 (covariates[a] + GFR)	Model 4 (covariates[a] + GFR+ Alb/Cr)
Alb/Cr				
Tertile 1	Reference	Reference	Reference	N/A
Tertile 2	1.34 (0.62, 2.88)	1.30 (0.60, 2.82)	1.47 (0.67, 3.19)	
Tertile 3	5.27 (2.73, 10.17)	2.74 (1.39, 5.41)	2.49 (1.24, 5.00)	
Per SD	3.45 (2.60, 4.57)	2.53 (1.83, 3.51)	2.30 (1.65, 3.19)	
KIM-1/Cr				
Tertile 1	Reference	Reference	Reference	Reference
Tertile 2	1.48 (0.84, 2.60)	1.03 (0.58, 1.83)	0.97 (0.54, 1.74)	0.66 (0.36, 1.21)
Tertile 3	1.38 (0.77, 2.47)	1.29 (0.70, 2.36)	1.17 (0.63, 2.16)	0.70 (0.37, 1.34)
Per SD	1.26 (0.98, 1.60)	1.18 (0.91, 1.53)	1.18 (0.90, 1.53)	0.95 (0.71, 1.28)
NAG/Cr				
Tertile 1	Reference	Reference	Reference	Reference
Tertile 2	2.33 (1.13, 4.77)	1.16 (0.54, 2.46)	0.99 (0.46, 2.11)	0.75 (0.34, 1.64)
Tertile 3	4.84 (2.48, 9.45)	2.47 (1.19, 5.14)	2.36 (1.13, 4.93)	1.34 (0.59, 3.05)
Per SD	2.30 (1.69, 3.13)	1.83 (1.24, 2.69)	1.74 (1.19, 2.54)	1.18 (0.80, 1.73)
NGAL/Cr				
Tertile 1	Reference	Reference	Reference	Reference
Tertile 2	1.18 (0.61, 2.27)	2.93 (1.37, 6.25)	3.25 (1.48, 7.13)	3.76 (1.67, 8.47)
Tertile 3	2.62 (1.46, 4.71)	7.79 (3.33, 18.20)	8.26 (3.50, 19.49)	6.88 (2.91, 16.31)
Per SD	1.44 (1.16, 1.80)	1.92 (1.46, 2.52)	1.93 (1.48, 2.52)	1.59 (1.20, 2.11)
L-FABP/Cr				
Undetected	Reference	Reference	Reference	Reference
Detected < median	0.54 (0.26, 1.13)	0.95 (0.44, 2.03)	0.91 (0.42, 1.97)	0.53 (0.24, 1.15)
Detected ≥ median	2.58 (1.57, 4.25)	1.91 (1.10, 3.30)	1.33 (0.73, 2.40)	0.40 (0.19, 0.83)

[a]Includes age, sex, duration of diabetes, hypertension, HbA_{1c} and study cohort

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Table 4

HRs and 95% CIs for all-cause mortality by normalised urinary biomarker

Urinary biomarker	All-cause mortality (*n*=80/260)			
	Model 1 (univariate)	Model 2 (covariates[a])	Model 3 (covariates[a] + GFR)	Model 4 (covariates[a] + GFR+ Alb/Cr)
Alb/Cr				
Tertile 1	Reference	Reference	Reference	N/A
Tertile 2	0.74 (0.43, 1.28)	0.64 (0.37, 1.12)	0.66 (0.38, 1.16)	
Tertile 3	1.65 (1.04, 2.63)	1.10 (0.65, 1.89)	1.07 (0.62, 1.85)	
Per SD	1.54 (1.25, 1.89)	1.23 (0.96, 1.59)	1.20 (0.92, 1.55)	
KIM-1/Cr				
Tertile 1	Reference	Reference	Reference	Reference
Tertile 2	1.12 (0.66, 1.89)	0.93 (0.55, 1.59)	0.93 (0.55, 1.60)	0.89 (0.51, 1.53)
Tertile 3	2.06 (1.28, 3.32)	1.54 (0.94, 2.52)	1.49 (0.91, 2.46)	1.38 (0.81, 2.33)
Per SD	1.30 (1.05, 1.62)	1.15 (0.92, 1.45)	1.15 (0.91, 1.45)	1.10 (0.86, 1.41)
NAG/Cr				
Tertile 1	Reference	Reference	Reference	Reference
Tertile 2	1.48 (0.87, 2.54)	1.20 (0.67, 2.16)	1.20 (0.67, 2.14)	1.16 (0.64, 2.09)
Tertile 3	2.23 (1.35, 3.69)	1.76 (0.98, 3.15)	1.71 (0.95, 3.08)	1.59 (0.85, 2.98)
Per SD	1.56 (1.20, 2.01)	1.40 (1.05, 1.86)	1.37 (1.03, 1.83)	1.33 (0.97, 1.82)
NGAL/Cr				
Tertile 1	Reference	Reference	Reference	Reference
Tertile 2	0.54 (0.33, 0.90)	1.10 (0.59, 2.06)	1.09 (0.58, 2.05)	1.09 (0.58, 2.05)
Tertile 3	0.90 (0.57, 1.40)	1.87 (0.99, 3.55)	1.78 (0.93, 3.39)	1.68 (0.87, 3.23)
Per SD	1.04 (0.85, 1.25)	1.44 (1.13, 1.85)	1.42 (1.11, 1.82)	1.39 (1.06, 1.82)
L-FABP/Cr				
Undetected	Reference	Reference	Reference	Reference
Detected < median	1.24 (0.74, 2.07)	1.25 (0.73, 2.14)	1.25 (0.73, 2.13)	1.15 (0.67, 1.98)
Detected ≥ median	1.97 (1.24, 3.12)	1.24 (0.75, 2.03)	1.11 (0.65, 1.88)	0.87 (0.46, 1.63)

[a] Includes age, sex, duration of diabetes, hypertension, HbA_{1c} and study cohort

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Table 5

c-statistics and *p* values for the likelihood ratio tests and the 13-year relative IDI for the categorical and continuous Cox proportional hazards models with and without the biomarker information

Outcome/model	c-statistic without biomarker[a]	c-statistic with biomarker[a]	Difference in c-statistic (95% CI)	Likelihood ratio *p* value	Relative IDI_{13}
ESRD					
KIM-1/Cr tertiles	0.828	0.829	0.001 (−0.005, 0.018)	0.389	0.015 (−0.007, 0.111)
KIM-1/Cr per 1 SD	0.828	0.828	0.000 (−0.004, 0.008)	0.725	0.005 (−0.008, 0.055)
NAG/Cr tertiles	0.828	0.830	0.002 (−0.005, 0.019)	0.169	0.005 (−0.009, 0.088)
NAG/Cr per 1 SD	0.828	0.828	0.000 (−0.005, 0.010)	0.397	−0.003 (−0.012, 0.031)
NGAL/Cr tertiles	0.828	0.835	0.007 (−0.007, 0.030)	<0.001	0.074 (0.003, 0.203)
NGAL/Cr per 1 SD	0.828	0.833	0.005 (−0.005, 0.025)	0.001	0.029 (−0.011, 0.124)
L-FABP/Cr[b]	0.828	0.832	0.004 (−0.006, 0.026)	0.042	0.033 (−0.002, 0.138)
All-cause mortality					
KIM-1/Cr tertiles	0.710	0.720	0.010 (−0.001, 0.036)	0.184	0.039 (0.001, 0.213)
KIM-1/Cr per 1 SD	0.710	0.709	−0.001 (−0.004, 0.020)	0.448	0.006 (−0.002, 0.106)
NAG/Cr tertiles	0.710	0.711	0.001 (−0.005, 0.026)	0.278	0.026 (−0.004, 0.178)
NAG/Cr per 1 SD	0.710	0.715	0.005 (−0.003, 0.034)	0.059	0.027 (−0.005, 0.172)
NGAL/Cr tertiles	0.710	0.716	0.006 (−0.003, 0.030)	0.210	0.033 (−0.001, 0.185)
NGAL/Cr per 1 SD	0.710	0.722	0.012 (−0.001, 0.039)	0.018	0.070 (0.001, 0.239)
L-FABP/Cr[b]	0.710	0.714	0.004 (−0.003, 0.022)	0.667	0.015 (−0.001, 0.161)

[a] Adjusted for age, sex, duration of diabetes, hypertension, HbA_{1C}, study cohort, GFR and Alb/Cr

[b] Categories are undetected vs detected <median vs detected ≥ median