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To cite this version:

João Pedro Ferreira, Patrick Rossignol, George Bakris, Cyrus Mehta, William B White, et al.. Blood and Urine Biomarkers Predicting Worsening Kidney Function in Patients with Type 2 Diabetes Post-Acute Coronary Syndrome: An Analysis from the EXAMINE Trial. American Journal of Nephrology, 2022, 52 (12), pp.969-976. 10.1159/000519436 . hal-03529807

HAL Id: hal-03529807

https://hal.univ-lorraine.fr/hal-03529807v1

Submitted on 17 Jan 2022

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Blood and urine biomarkers predicting worsening kidney function in patients with type 2 diabetes post-acute coronary syndrome: an analysis from the EXAMINE trial

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Abstract

Introduction: Worsening kidney function (WKF) is frequent among patients with type 2 diabetes (T2D) and a recent acute coronary syndrome (ACS) and is associated with a poor prognosis. An accurate prediction of WKF is clinically important.

Aims: Using data from the EXAMINE trial including patients with T2D and a recent ACS, and a large biomarker panel incorporating proteins measured both in blood and urine, we aim to determine those with best performance for WKF prediction.

Methods: WKF was defined as a ≥40% eGFR drop from baseline, eGFR<15ml/min, or dialysis. Mixed effects and time-updated Cox models were used.

Results: 5131 patients were included from whom 222 (4.3%) developed at least one WKF episode over a median follow-up of 18 months. Patients who developed WKF were more frequently women, had longer diabetes duration, a more frequent HF history, higher anemia prevalence, and impaired kidney function. In multivariable models including all variables (clinical and biomarkers) independently associated with WKF with a P-value≤0.0001, blood KIM-1 was (by far) the variable with strongest WKF association, followed by anemia. KIM-1 alone provided good discrimination for WKF prediction (AUC=0.73). Patients in the high KIM-1-derived risk tertile had a 6.7-fold higher risk of any WKF than patients classified as low risk. In time-updated Cox models, the occurrence of WKF was independently associated with a higher risk of death: adjusted HR=4.93 (3.06-7.96), P-value<0.0001.

Conclusion: Blood KIM-1 was the biomarker the strongest association with WKF. The occurrence of WKF was independently associated with a higher risk of subsequent cardiovascular events and mortality.

Key-words: kidney injury molecule 1, worsening kidney function, type 2 diabetes, acute coronary syndromes, prognosis.

Introduction

Worsening kidney function (WKF) is frequent among patients with type 2 diabetes (T2D) and is associated with a poor prognosis.[1] The risk of WKF is particularly elevated in patients with T2D with a very high cardiovascular risk, such as those with a recent acute coronary syndrome (ACS) or myocardial infarction (MI).[2, 3]

Blood and urine biomarkers reflecting kidney damage have been shown to predict WKF and cardiovascular events in patients with and without T2D.[4-8] However, the identified biomarkers vary largely between studies, which poses difficulties in selecting biomarkers for clinical implementation.[9]

The use of large biomarker panels incorporating both urinary and blood biomarkers may help in selecting the biomarkers with the best performance in predicting WKF. An accurate identification of patients at higher risk for WKF is important to select those who might experience a greater benefit from therapies that reduce kidney events and improve cardiovascular outcomes in patients with T2D.[10-13]

Using data from the Cardiovascular Outcomes Study of Alogliptin in Patients with Type 2 Diabetes and Acute Coronary Syndrome (EXAMINE) trial,[14] and a large biomarker panel incorporating proteins measured both in blood and urine, we aim to determine those with best performance for prediction WKF.

Methods

Study design

Details of the EXAMINE trial design have been previously published[14, 15]. In short, a total of 5380 patients with T2D who had a recent ACS were randomly assigned to either alogliptin or placebo.

In the overall population, alogliptin significantly reduced glycated hemoglobin (HBA1c) without increasing the rates of major adverse cardiovascular events over a median follow-up of 18 months.

The steering committee, consisting of academic members and three nonvoting representatives of the sponsor (Takeda Development Center Americas), designed and oversaw the conduct of the trial. An independent data and safety monitoring committee monitored the trial and had access to the unblinded data.

For the present analysis we included 5131 patients (95.4% of the overall population) who had biomarkers available.

Study patients

Patients were eligible for enrolment if they had received a diagnosis of T2D, were receiving antidiabetic therapy (other than a DPP-4 inhibitor or GLP-1 analogue) and had an ACS (MI and unstable angina requiring hospitalization) within 15 to 90 days prior to randomization. Major exclusion criteria were a diagnosis of type 1 diabetes, unstable cardiac disorders (e.g., advanced heart failure, refractory angina, uncontrolled arrhythmias, critical valvular heart disease, or severe uncontrolled hypertension), and dialysis within 14 days before screening.

Institutional Review Board approval was obtained, and all patients provided informed consent to participate in the trial. The EXAMINE trial is registered with the clinicaltrials.gov number NCT00968708.

End points

The primary endpoint in EXAMINE was a composite of death from cardiovascular causes, nonfatal MI, or nonfatal stroke, adjudicated by an independent committee.

In the present analysis, we defined WKF as % of change in estimated glomerular filtration rate (eGFR) at each study visit (month 1, 3, 6, 9, 12 and every 4 months up to month 32; after month 32 eGFR had >90% of values missing) relative to the randomization visit. An eGFR drop ≥40% from randomization, or <15 ml/min/1.73m^2, or progression to dialysis was considered WKF. A WKF that persisted in two or more consecutive visits was considered a sustained worsening kidney function (sWKF). We used eGFR as calculated by the CKD-EPI formula.[16]

Additionally, we studied the association of WKF with subsequent occurrence of the study the primary endpoint, and also the occurrence of the composite of heart failure (HF) hospitalization or cardiovascular death, plus all-cause mortality.

Clinical and biomarker measurements

Medical history and a physical examination were all recorded at randomization. A large protein biomarker panel with 92 biomarkers from a wide range of pathophysiological domains was measured (Olink® CVDII panel) at randomization. We selected this panel because it contains known human cardiovascular and inflammatory markers as well as some exploratory human proteins which may have potential as new markers of cardiovascular disease. These proteins are involved in multiple disease processes such as immune/inflammatory response, angiogenesis, coagulation, and cell adhesion. In short, the biomarkers were measured using a high-throughput technique using the Olink Proseek® Multiplex CVDII 96x96 kit, which simultaneously measures 92 proteins in 1µl of plasma. The kit uses a proximity extension assay technology, where 92 oligonucleotide-labelled antibody probe pairs bind to their respective target present in the sample. The platform provides Log2 normalized protein expression (NPX) data wherein a high protein value corresponds to a high protein concentration, but not an absolute quantification.

The full proteins names from the CVDII panel are described in the *Supplemental Table 1*. For further details consult https://www.olink.com/.

In addition to the 92 Olink® biomarkers, high-sensitivity troponin I (hsTnI; troponin) was measured in plasma samples with the ARCHITECT i2000SR analyser (Abbott Laboratories®); urinary neutrophil gelatinase-associated lipocalin (uNGAL) was measured by enzyme immunoassay (R&D systems); and urinary kidney injury molecule-1 (uKIM-1) was measured by a microbead ELISA (R&D systems). Both uNGAL and uKIM-1 were indexed to urinary creatinine as recommended by the National Institute for Diabetes and Digestive and Kidney Diseases CKD biomarkers consortium.[17]

Statistical analyses

Baseline characteristics of the patients were compared between those who developed and those who did not develop WKF throughout the follow-up by means of chi-square tests for categorical variables, t-tests for continuous normally distributed variables, and Wilcoxon rank sum tests for continuous non-normally distributed variables. Mixed effects models were used to test the association between the biomarker levels and the occurrence of WKF (mixed effects logistic regression), and the change in eGFR slope (mixed effects linear regression). All mixed effects models were adjusted on age, sex, baseline eGFR, anemia, diabetes duration, smoking, hypertension, prior HF hospitalization, use of diuretics, insulin, metformin, statins, randomized treatment assignment (alogliptin or placebo), study visit, and randomized treatment by study visits interactions; random effects were assigned to patients in panel data with unstructured covariance matrix to allow for correlation between repeated measures. The associations between biomarker levels and WKF (or eGFR slope) were studied in multivariable models fully adjusted on the

variables described in the previous step. To select the biomarker(s) with the strongest WKF predictive power while keeping the models parsimonious, we tested each individual biomarker while adjusting for the full list of covariates described previous step, and we retained only the biomarkers associated with WKF with a P-value of 0.0001 (1/10000) or less, which is a stricter selection than the conventionally used Bonferroni correction that would have given a P-value of 0.0005 or less (95/0.05 =0.0005). After this “pre-selection” process, all variables/biomarkers with a P-value ≤0.0001 were entered in a multivariable stepwise forward selection model with a P-value of less than 0.05 used to be retained in the model and we computed the area under the curve (AUC) of the model. After the stepwise selection, we then kept the variables with P-value ≤0.0001 from the model while comparing the AUC until we reach the simplest and final model without much compromise of the model’s accuracy. Afterwards, the linear predictor derived from the final model was divided in tertiles, and WKF events were plotted by tertiles of predicted risk. The association of WKF with subsequent cardiovascular outcomes was studied in time-updated Cox models, both crude and adjusted for the variables above described. All statistical analyses were performed with Stata® (StataCorp. 2019. Stata Statistical Software: Release 16.1. College Station, TX: StataCorp LLC).

Results

Baseline patients’ characteristics by worsening kidney function status

Patients who developed at least one WKF episode during the follow-up (n =222; 4.3%) were more frequently women (43 vs. 32%), had longer diabetes duration (10 vs. 7 years), were more frequently treated with insulin (41 vs. 30%), and less frequently treated with metformin (55 vs. 66%), smoked less (10 vs. 14%), had a

more frequent history of HF (46 vs. 28%), higher prevalence of anemia (47 vs 24%), more frequent kidney function impairment (eGFR <60: 46 vs. 28%), higher blood pressure (SBP >140: 26 vs. 20%), and were more frequently treated with diuretics (60 vs. 37%), and less frequently treated with statins (85 vs. 91%). *Table 1*.

Associations with worsening kidney function and eGFR slope changes

Alogliptin (vs. placebo) had no statistically significant impact on eGFR slope of change during the follow-up: -1.11 (-2.55 to 0.33) ml/min, P =0.13. *Supplemental Figure 1*.

In multivariable models, the strongest clinical variable associated with WKF was anemia: β =1.39 (0.96 to 1.82), Z =6.4. *Supplemental Table 2*. In biomarkers (adjusted for clinical variables) the one with strongest association with WKF was blood KIM-1: β =0.84 (0.66 to 1.03) per Log2 increase, Z =9.1; followed by platelet growth factor (PGF), Z =7.1; and TRAIL receptor 2 (TRAILR2), Z =6.9. A total of 18 biomarkers were independently associated with WKF with a P-value ≤0.0001 (Z ≤3.8). Urinary KIM-1 was associated with WKF but with a much weaker association than blood KIM-1, Z =2.9 for urinary KIM-1. All biomarkers listed by descending order of strength of association with WKF are listed in the *Supplemental Table 3*. All the biomarkers associated with WKF were also associated with eGFR slope deterioration. *Supplemental Table 4*.

In multivariable models including all the variables (clinical and biomarkers) independently associated with WKF with a P-value ≤0.0001, blood KIM-1 was (by far) the variable with strongest association with WKF, followed by anemia. KIM-1 alone provided good discrimination for the prediction of WKF (AUC =0.73), while anemia alone provided only moderate discrimination and much lower than KIM-1

alone (AUC =0.65). *Table 2*. Urinary albumin-to-creatinine ratio (UACR) variable contains 42% (n =2245) missing values. As sensitivity analysis, we have adjusted our models to UACR (plus age, sex, and eGFR) in complete cases (n =2999) and after multiple imputation (n =5380), and KIM-1 remained the variable with the strongest association with the occurrence of WKF. *Supplemental Table 5*.

Worsening kidney function events by KIM-1 levels

The predicted risk of WKF derived from KIM-1 alone was divided in tertiles (low, intermediate/medium, and high risk). Patients in the high risk tertile had a 6.7-fold higher risk of any WKF than patients classified as low risk, and the risk of sWKF was 12.5-fold higher in high risk compared with low risk. *Table 3 & Figure 1*.

Events after worsening kidney function

In time-updated Cox models, the occurrence of WKF was independently associated with a higher risk of experiencing a subsequent primary outcome event: adjusted HR =4.25 (2.83-6.39); cardiovascular death or HF hospitalization: adjusted HR =6.19 (4.09-9.36); and all-cause mortality: adjusted HR =4.93 (3.06-7.96) (all P <0.0001). *Figure 2*.

Discussion

The present study shows that in more than 5000 patients with T2D and a recent ACS in whom 95 biomarkers (in both urine and blood) had been measured at baseline, blood KIM-1 was the biomarker with the strongest association with WKF. The occurrence of WKF was independently associated with a higher risk of subsequent cardiovascular events and mortality.

Identifying patients more likely to develop kidney dysfunction is important both for clinical practice and research. To date, the prediction of WKF is often performed by detecting rapid rises in serum creatinine or rapid decreases in eGFR, which is far from ideal, because it frequently represents overt WKF resulting in a late diagnosis, often at advanced stages of kidney injury where therapeutic options are limited and the prognosis is poor.[18] Therefore, more important is predicting the occurrence of WKF before its onset, so that preventive strategies can be implemented.[19] In this regard, several molecules, measured both in blood and urine, have been identified as potential markers for early detection of kidney damage before rises in serum creatinine or eGFR decline.[20-23] However, these markers are not routinely used in clinical practice nor for patient selection in clinical trials, due to limitations in accuracy and reproducibility.[3, 6]

In our study, blood KIM-1 was the biomarker the strongest association with WKF and was also strongly associated with eGFR slope deterioration over time. KIM-1 is upregulated in the proximal tubular cells following kidney injury.[24] The disruption of renal endothelial cells, with loss of cellular adhesion junctions and disruption of extra-cellular matrix, facilitates KIM-1 movement into the circulation and its blood detection, beyond its urinary excretion. In fact, raised levels of blood KIM-1 may represent a more severe kidney injury due to the disruption of the endothelial barrier.[25] Higher levels of blood KIM-1 have been shown to predict acute and chronic kidney injury in patients with type 1 diabetes,[4, 26] long-term deterioration of kidney function in the general population,[27] and poorer renal function in HF.[28] In a study including patients with type 1 diabetes without proteinuria in whom both blood and urine KIM-1 were measured, blood KIM-1 (but not urine KIM-1) was the biomarker more strongly associated with kidney function decline.[26] In a previous analysis of the

EXAMINE trial studying urinary KIM-1 and NGAL not measuring blood biomarkers, neither urinary KIM-1 nor urinary NGAL improved the prediction of eGFR decline.[29] These findings were concordant with the present analysis, where both blood and urine KIM-1 were measured, but blood KIM-1 had a stronger association with WKF and renal function “slope” decline than urinary KIM-1, urinary NGAL or any other measured biomarker. Our results suggest that damage to proximal tubules with disruption of the endothelial barrier plays an independent role in the development and progression of kidney dysfunction.

Important clinical and research implications can be drawn from these findings. Due to extensive replication and strong prediction capacity, blood KIM-1 could be implemented for the prediction of kidney function decline in the clinical setting, allowing to individualize preventive strategies to patients at higher risk of kidney dysfunction progression. These preventive strategies might include treatment with sodium glucose co-transporter 1/2 inhibitors and finerenone, that have been shown to reduce WKF and prevent kidney function decline in patients with and without diabetes.[10-13, 30, 31] Interestingly, empagliflozin has been shown to reduce the urinary levels of KIM-1, suggesting that empagliflozin may reduce tubular injury and improve the function of the proximal tubule.[32]

Blood KIM-1 can also be used in clinical trials for risk enrichment of kidney outcomes, allowing to increase the number of events and the study power, tailored to patients who might benefit the most from the treatment being studied.[33] Furthermore, along with brain natriuretic peptides and troponin, that strongly predict cardiovascular events,[34] the addition of KIM-1 for WKF prediction, may allow an optimal risk assessment of cardio-kidney endpoints.[33]

Limitations

Some limitations should be acknowledged in this study. This is a post-hoc analysis of a randomized controlled trial subject to biases inherent to observational data. Blood KIM-1 and all the Olink® biomarkers represent normalized concentrations and cannot be translated into cut-points ready for clinical use. Further studies using other laboratorial techniques for measuring blood KIM-1 (e.g., ELISA) are warranted to validate our findings. Despite extensive replication of blood KIM-1 as a biomarker strongly associated with WKF in several populations, further data are needed for patients with T2D and an ACS, where our study is unique. Urinary albumin-to-creatinine ratio has 42% of missing values in EXAMINE; still in sensitivity analysis adjusting for urinary albumin-to-creatinine ratio (plus age, sex, and eGFR), KIM1 remained the variable with strongest associated with WKF.

Conclusions

In patients with T2D and a recent ACS, blood KIM-1 was the biomarker with the strongest association with WKF. The occurrence of WKF was independently associated with a higher risk of subsequent cardiovascular events and mortality. Further studies are needed to confirm these hypothesis generating findings and assess whether KIM-1 could be implemented for early prediction of kidney outcomes in the clinical setting.

Availability of Data and Materials

The data and materials may be available upon reasonable request.

Funding

The EXAMINE study was funded by Takeda but the present analysis did not receive funding.

Disclosures

Dr Ferreira is a consultant for Boehringer-Ingelheim, and receives research support from AstraZeneca and Novartis. Dr Rossignol reports personal fees from Ablative Solutions, AstraZeneca, Bayer, Boehringer-Ingelheim, Corvidia, CVRx, Fresenius, G3P (stocks), Grunenthal, Idorsia, KBP, Novartis, NovoNordisk, Relypsa, Sanofi, Sequana Medical, Servier, Stealth Peptides, Vifor, Vifor Fresenius Medical Care Renal Pharma; and Cofounder: CardioRenal, a company developing sensors for the home monitoring of potassium and creatinine. Dr Zannad reports personal fees from Boehringer Ingelheim during the conduct of the study; personal fees from Janssen, Novartis, Boston Scientific, Amgen, CVRx, AstraZeneca, Vifor Fresenius, Cardior, Cereno pharmacuetical, Applied Therapeutics, Merck, Bayer, and Cellprothera outside the submitted work; and other support from CVCT and Cardiorenal, outside the submitted work. Dr Bakris, has received personal fees from Takeda Development Center, is a consultant for Merck, Relypsa, and is on the steering committee for international renal/CV outcomes trials for Janssen, Bayer, Vascular Dynamics. Dr White has received research support from the National Institute of

Aging (NIH) and personal fees from Takeda Development Center (Deerfield, IL, USA) during the conduct of the EXAMINE trial until 2016 (Steering Committee Chair). All the other authors reported not having potential conflicts of interest relevant to this article.

Acknowledgments

EXAMINE is a clinical trial sponsored by Takeda Global Research and Development Center, Inc., Deerfield, IL. The authors are solely responsible for the design of the study, all study analyses, and the drafting and editing of the manuscript and its final contents.

Clinical trials identifier: Clinicaltrials.gov-NCT00968708.

JPF, PR, FZ are supported by a public grant overseen by the French National Research Agency (ANR) as part of the second “Investissements d’Avenir” programme (ANR-15-RHU-0004). All the other authors have nothing to disclose with regards to this manuscript.

JPF wrote the manuscript, performed the statistical analysis and made critical revisions; CM contributed to the oversight of the EXAMINE trial, as a steering committee member and to discussion and reviewed/edited the manuscript including the revision of the statistical methods and analysis; GB contributed to the oversight of the EXAMINE trial, as a steering committee member and reviewed/edited the manuscript; PR reviewed/edited the manuscript; WBW contributed to the oversight of the EXAMINE trial, as the principal investigator of the trial, and reviewed/edited the manuscript; FZ contributed to the oversight of the EXAMINE trial, as a steering committee member discussion and reviewed/edited the manuscript.

Dr. João Pedro Ferreira is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Table 1. Baseline patients' characteristics by worsening kidney function status

Variable	No WKF	WKF	P
N. =5131	4909	222	
Age (years)	60.9 ± 9.9	61.8 ± 10.1	0.19
Age >65yr	1735 (35.3%)	88 (39.6%)	0.19
Male	3359 (68.4%)	127 (57.2%)	<0.001
Duration of T2D (years)	7.0 (2.6, 13.5)	10.4 (4.0, 17.4)	<0.001
HBA1c (%)	8.0 ± 1.1	8.0 ± 1.0	0.66
Insulin	1451 (29.6%)	90 (40.5%)	<0.001
Metformin	3265 (66.5%)	122 (55.0%)	<0.001
Sulfonylureas	2287 (46.6%)	97 (43.7%)	0.40
Thiazolidinediones	117 (2.4%)	9 (4.1%)	0.12
BMI (kg/m2)	29.5 ± 5.5	29.8 ± 6.4	0.37
Race			0.33
White	3580 (72.9%)	160 (72.1%)	
Asian	997 (20.3%)	41 (18.5%)	
Black	187 (3.8%)	10 (4.5%)	
Other	145 (3.0%)	11 (5.0%)	
Smoker (current)	683 (13.9%)	21 (9.5%)	0.059
MI (history)	4320 (88.0%)	191 (86.0%)	0.38
HF (history)	1369 (27.9%)	103 (46.4%)	<0.001
Stroke (history)	344 (7.0%)	20 (9.0%)	0.26
PAD (history)	461 (9.4%)	27 (12.2%)	0.17
Atrial Fibrillation	343 (7.0%)	17 (7.7%)	0.70
Index event MI	3780 (77.2%)	169 (76.5%)	0.81
Index event ACS	1118 (22.8%)	52 (23.5%)	
Hemoglobin (g/dL)	13.5 ± 1.6	12.6 ± 1.6	<0.001
Anemia	1184 (24.2%)	104 (46.8%)	<0.001
eGFR (ml/min/1.73 m2)	71.2 ± 21.1	63.9 ± 27.0	<0.001
eGFR <60	1383 (28.2%)	102 (45.9%)	<0.001
Heart rate (bpm)	71.4 ± 10.8	72.3 ± 12.1	0.19
SBP (mmHg)	128.9 ± 16.6	132.3 ± 17.5	0.003
SBP >140	983 (20.0%)	58 (26.1%)	0.027
Antiplatelet agents	4773 (97.2%)	218 (98.2%)	0.39
Beta-blockers	4034 (82.2%)	183 (82.4%)	0.92
ACEi or ARB	4054 (82.6%)	179 (80.6%)	0.45
Calcium channel blockers	1084 (22.1%)	58 (26.1%)	0.16
Diuretics	1792 (36.5%)	133 (59.9%)	<0.001
Statins	4462 (90.9%)	189 (85.1%)	0.004
Randomized to alogliptin	2456 (50.0%)	126 (56.8%)	0.050

Legend: WKF, worsening kidney function (first occurrence); T2D, type 2 diabetes; HBA1c, glycated hemoglobin; BMI, body mass index; MI, myocardial infarction; HF, heart failure; PAD, peripheral artery disease; ACS, acute coronary syndrome; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; ACEi or ARB, angiotensin converting enzyme inhibitors or angiotensin receptor blockers.

Table 2. Multivariable stepwise models predicting worsening kidney function

Biomarker	**Beta coef. (95%CI)**	**Z-score**	**P-value**
Model 1			
KIM-1	0.50 (0.36 to 0.65)	6.6	<0.00001
Anemia	0.70 (0.36 to 1.04)	4.0	0.00005
VEGFD	0.76 (0.32 to 1.20)	3.4	0.001
IL4RA	0.50 (0.21 to 0.80)	3.3	0.001
uNGAL	0.11 (0.03 to 0.19)	2.8	0.006
SPON2	-0.88 (-1.64 to -0.13)	-2.3	0.022
AUC =0.78	-	-	-
Model 2			
KIM-1	0.78 (0.63 to 0.92)	10.6	0.00001
Anemia	0.90 (0.54 to 1.26)	4.9	0.00001
AUC =0.76	-	-	-
Model 3 *			
KIM-1	0.78 (0.66 to 0.89)	12.9	0.00001
AUC =0.73	-	-	-

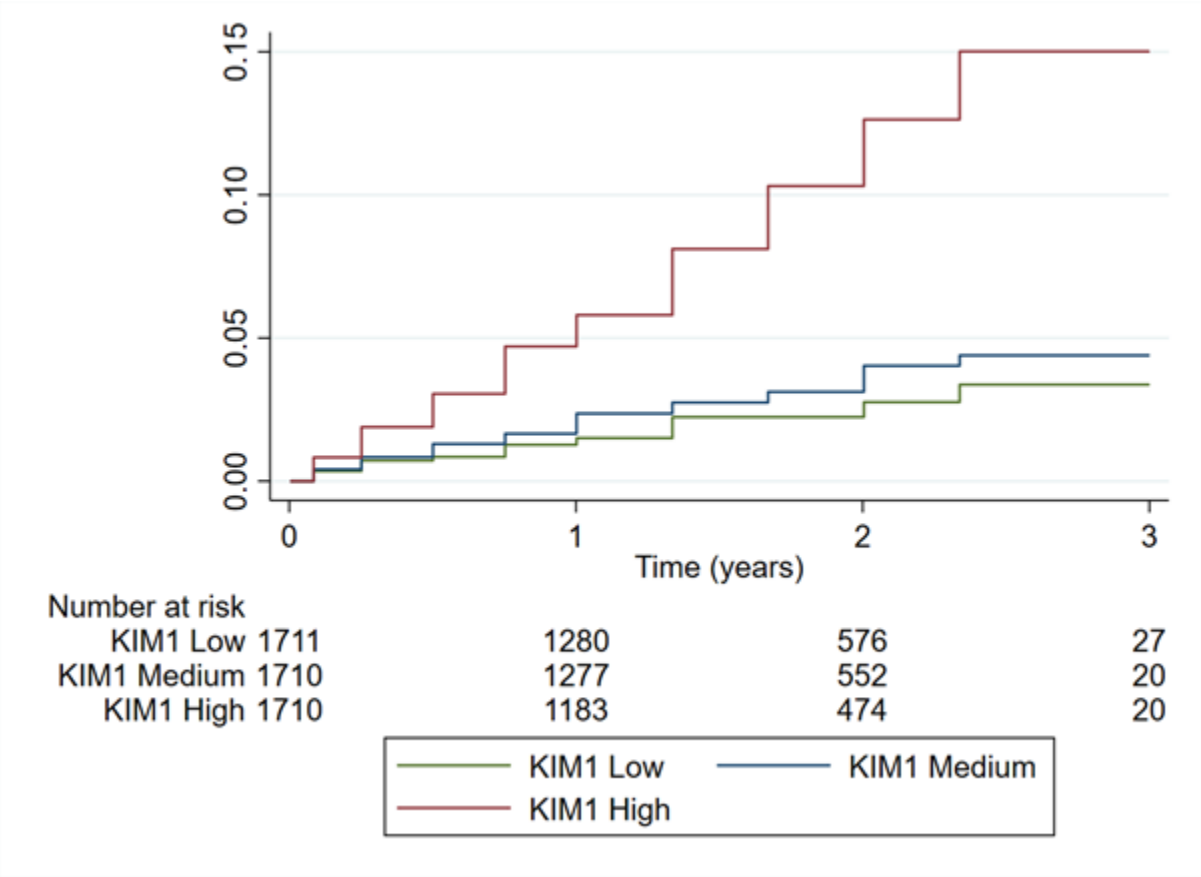
Legend: KIM-1, kidney injury molecule 1; VEGFD, vascular endothelial growth factor-D; IL4RA, interleukin 4 receptor alfa subunit; uNGAL, urinary neutrophil gelatinase-associated lipocalin; SPON2, spondin 2.
The correlation coefficient of KIM-1 with the other proteins was not greater than 0.5.
Model 1 shows the results of the stepwise forward model that started with all the variables with a P-value ≤0.0001 shown in the Supplemental Tables 2 & 3 (light green), the retained variables with a P-value of <0.05 to stay in the model are shown in the Model 1 of this table; Model 2 shows the results after keeping only the variables with a P-value ≤0.0001 in Model 1; Model 3 is a simplest model retaining only KIM-1, the variable with strongest association with worsening kidney function.
*Anemia alone gave an AUC =0.65.

Table 3. Worsening kidney function events by tertiles of risk predicted from blood KIM-1 levels

KIM-1 predicted risk	**Low**	**Intermediate**	**High**	**Total**
Total WKF (first and recurrent)				
No WKF	11455	11471	11217	34143
WKF	44 (0.4)	58 (0.5)	216 (1.9)	318
OR (95%CI)	Ref.	1.40 (0.83-2.36)	6.68 (4.15-10.76)	-
AUC =0.73	-	-	-	-
First WKF				
No WKF	1675	1662	1572	4909
WKF	36 (2.1)	48 (2.8)	138 (8.1)	222
HR (95%CI)	Ref.	1.37 (0.89-2.11)	4.2 (2.91-6.06)	-
C-index =0.69	-	-	-	-
sWKF				
No WKF	1707	1700	1644	5071
WKF	4 (0.2)	10 (0.6)	46 (2.7)	60
HR (95%CI)	Ref.	2.55 (0.80-8.13)	12.46 (4.48-34.60)	-
C-index =0.80	-	-	-	-

Legend: WKF, worsening kidney function; sWKF, sustained worsening kidney function; KIM-1, kidney injury molecule 1; OR, odds ratio; HR, hazard ratio; 95%CI, 95% confidence interval; AUC, area under the curve.
Low, intermediate, and high represent the tertiles of predicted risk from KIM-1 levels. KIM-1 predicted the occurrence of WKF in an incremental manner, regardless of the WKF definition.

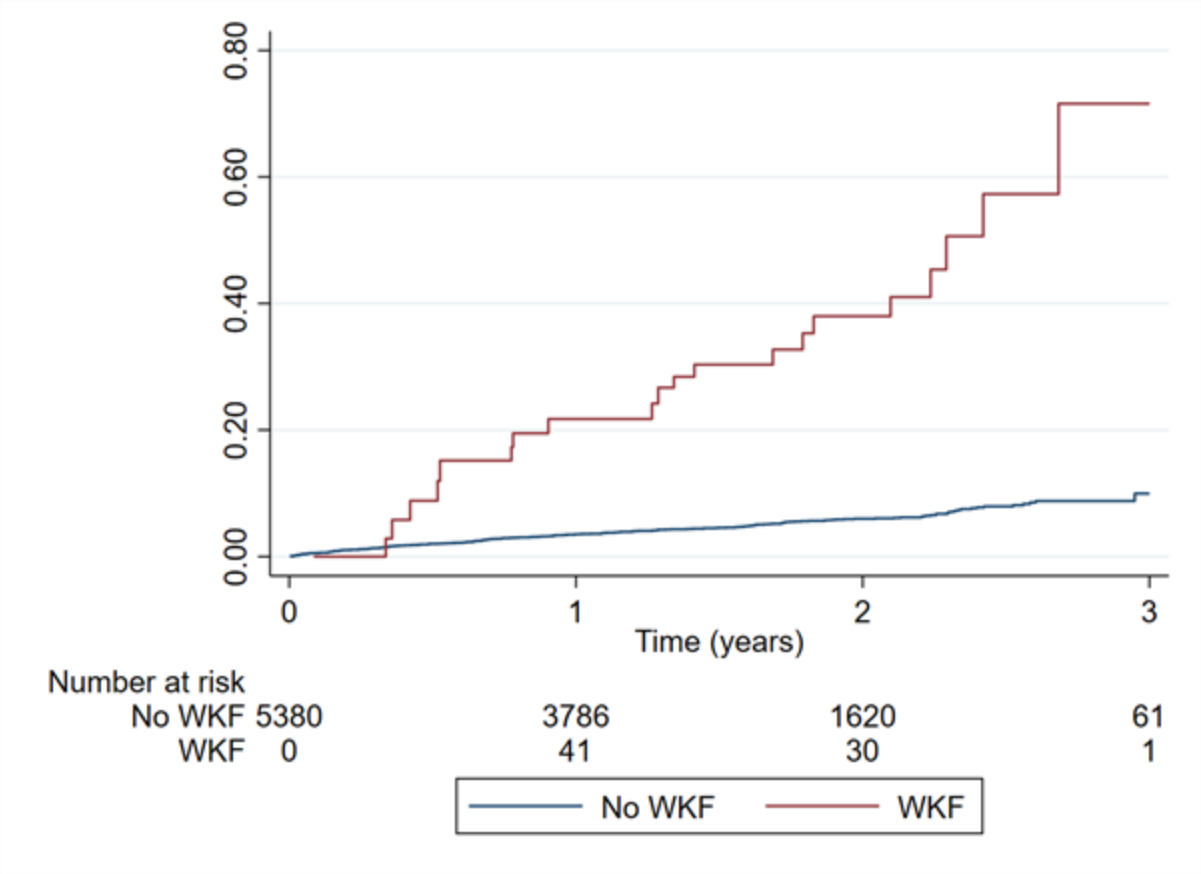
Figure 1. Worsening kidney function events by tertiles of risk predicted from blood KIM-1 levels



Legend: KIM-1, kidney injury molecule 1.

Caption: Higher KIM-1 levels predict worsening kidney function events defined by a deterioration in eGFR ≥40% from baseline.

Figure 2. Death after a worsening kidney function event



Legend: WKF, worsening kidney function defined by a deterioration in eGFR ≥40% from baseline.

Caption: Crude HR (95%CI) =8.05 (5.08-12.76), P <0.0001.