Serum protein biomarkers for estimated GFR decline – validated by iohexol clearance

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Significance statement

In search of biomarkers for GFR decline, estimated GFR (eGFR) from creatinine, cystatin C, or both have mostly been used. Whether the relationships between biomarkers and eGFR decline are similar to associations with measured GFR (mGFR) decline has not been investigated. This study revealed that some biomarkers showed statistically significant different associations with eGFR decline compared to mGFR decline, particularly for eGFR from cystatin C. The study indicates that non-GFR-related factors influence the relationship between biomarkers and eGFR decline. It concludes that the results of biomarker studies using eGFR, particularly eGFRcys, should be interpreted with caution.

Abstract

Background:

Several serum protein biomarkers have been proposed as risk factors for GFR decline using estimated GFR (eGFR) from creatinine or cystatin C. We investigated whether eGFR can be used as a surrogate endpoint for measured GFR (mGFR) when searching for biomarkers associated with GFR decline.

Methods:

In the Renal Iohexol Clearance Survey (RENIS), GFR was measured with plasma iohexol clearance in 1627 individuals without diabetes, kidney, or cardiovascular disease at baseline. After 11 years of follow up, 1409 participants had one or more follow-up GFR measurements. Using logistic regression and interval-censored Cox regression, we analyzed the association between baseline levels of 12 serum protein biomarkers with the risk of accelerated GFR decline and incident CKD for both mGFR and eGFR.

Results:

Several biomarkers exhibited different associations with eGFR decline compared to their association with mGFR decline. More biomarkers showed different associations with eGFRcys decline than with eGFRcre decline. Most of the different associations of eGFR decline vs mGFR decline remained statistically significant after adjustment for age, sex, and BMI, but several were attenuated and not significant after adjusting for the corresponding baseline mGFR or eGFR.

Conclusion:

In studies of some serum protein biomarkers, eGFR decline may not be an appropriate surrogate outcome for mGFR decline. Although the differences from mGFR decline are attenuated by adjustment for confounding factors in most cases, some persist. Proposed biomarkers from studies using eGFR should preferably be validated with mGFR.

Introduction

The prevalence of chronic kidney disease (CKD) is increasing worldwide.[1,2] Several studies have therefore investigated biomarkers to identify people at risk for CKD as well as the underlying mechanisms for GFR loss.

Biomarkers related to inflammation, immunity, fibrosis development, cell proliferation, angiogenesis, and apoptosis have been associated with loss of GFR in general population studies.[3-13] These studies used equations based on creatinine and/or cystatin C to estimate GFR (eGFR) and eGFR change rates. This method may introduce confounding and spurious associations between the biomarkers and renal outcomes because eGFR is biased by non-GFR-related factors such as diet, muscle mass, inflammation, obesity, and cardio-metabolic risk factors.[14-19] Some of these studies included persons with diabetes, cardiovascular disease (CVD), and other comorbidities, potentially increasing the problem of confounding.

The aim of this study was to investigate whether eGFR can be used as a surrogate endpoint for measured GFR (mGFR) decline when searching for biomarkers for GFR decline. We assessed the validity of associations between protein biomarkers and eGFR decline relative to the decline in measured GFR (mGFR). This was done by comparing the associations between 12 protein biomarkers measured at baseline and loss of kidney function assessed by eGFR from creatinine and cystatin C versus the measured GFR. We analyzed associations of protein biomarkers and risk of accelerated GFR decline and incident CKD using eGFR and mGFR in a representative sample of a middle-aged general population without self-reported diabetes, CVD, or kidney disease at baseline, where iohexol clearance had been measured three times during 11 years of follow-up.

Methods

Subjects

The Renal Iohexol Clearance Survey in Tromsø 6 (RENIS-T6) (2007-2009) was a substudy of the sixth Tromsø Study (T6), a general population survey in the municipality of Tromsø, Northern Norway.[20] In total, 2825 participants from T6 between 50-62 years of age without self-reported diabetes, kidney disease or CVD were invited to RENIS-T6. The response rate was 75%, and 1627 persons were investigated according to a predetermined study target size (Figure 1).[16] Of these 1627, 1324 had a follow-up in the RENIS follow-up (RENIS-FU) (2013-2015) and 1174 in RENIS-3 (2018-2020) after a median of 5.6 (interquartile range (IQR): 4.3-7.0) and 11 (IQR: 10.6-11.5) years, respectively, leaving 1410 participants with at least one follow-up GFR measurement and a total of 4213 GFR measurements.[21] Another 210 participants were included in RENIS 3,[21] these were not included in the current study because they did not have their GFR or proteins measured at baseline. The study was approved by the Norwegian Data Inspectorate and the Regional Ethics Committee of Northern Norway, and all participants provided written informed consent.

Iohexol clearance

GFR was measured using single sample plasma clearance of iohexol (Omnipaque, 300 mgI/ml; Amersham Health, London, UK)[22] in all three surveys; details have been reported previously.[23-25] The mean coefficient of variation for intraindividual mGFR variation was 4.2% in RENIS-FU (95% confidence interval (CI) 3.4-4.9%).[24] Iohexol concentration was measured using high-performance liquid chromatography (HPLC) in RENIS-T6 and RENIS-FU and by liquid chromatography–mass spectrometry (LC–MS) in RENIS-3. A calibration equation for the conversion of results between HPLC and LC–MS was developed, and the equation development has been described in detail.[21,26]

Serum protein biomarker selection and measurement

We selected serum protein biomarkers that have been associated with an increased risk of accelerated eGFR loss, CKD development, and renal aging in previous studies. The biomarkers were

selected based on a structured literature search of published articles during the last five years. Two researchers conducted the search using the PubMed database from March to April 2018, applying three slightly different searches to expand the search (listed in Table S1). Studies with diabetes type 1 and acute kidney injury patients were excluded. In total, 72 different proteins were identified from original research and review articles, mainly from longitudinal general population studies but also studies involving patients with type 2 diabetes, of which each researcher listed 20 proteins based on relevance in findings. We selected proteins which associated with different pathophysiological pathways to investigate a broad range of biomarkers associated with eGFR loss. Thirteen proteins available for analysis using the Luminex assay were selected (Figure S1). Full names of the 13 proteins are listed in Table 1, and abbreviations are used hereinafter.

Fasting serum samples collected at baseline and stored at -80 °C were thawed for protein measurement. TNFR2 was analyzed in 2015 using a quantitative sandwich ELISA with a QuantiKine kit from R&D systems, Inc. (Minneapolis, MN) as part of a previous project.[27] The other 12 proteins were analyzed between June 2018 and January 2019 using Luminex xMap multiplex technology (Bio-Plex 200 systems, BIO-RAD) and human magnetic bead-based assays from R&D Systems (Bio-Teche), consisting of microplates with 96-well plates and magnetic antibody-coated beads. All the microplates and accompanying standard solutions were from the same batch to avoid batch differences.

Due to differences in the required dilution factor of serum samples prior to protein analyses with Luminex technology, we used two separate kits. The first 9 proteins (CD40Lig, GDF-15, MCP-1, Tie-2, TRAIl-R2, FABP4, KIM-1, MMP7, and suPAR) were measured at a 1:2 dilution, and the last three (Umod, Gal-3, and MMP2) were measured at a 1:50 dilution. Protein levels were calculated using a five-parameter logistic (5-PL) standard curve and displayed as the mean of duplicate measurements. KIM-1 was undetectable (below the lower limit of detection) in all but 37 of the baseline samples and

was thus excluded from the analysis. Intra- and interassay CVs were between 2.9–6.4% and 4.9–19.3% respectively (Table S2).

Of the selected biomarkers, two markers of fibrosis (MMP7 and MMP2) as well as TNFR2 have been included in previous publications from the first RENIS follow-up after a median of 5.6 years (RENIS-FU).[14,27,28]

Other study variables

Information on medication use and smoking habits (current, previous, or never daily smoker) was collected through a questionnaire. Height and weight were measured to calculate body mass index (BMI: weight in kg divided by height in meters squared). Blood pressure (BP) was measured three times at one-minute intervals after 2 minutes of rest using an automated device (model UA 799; A&D, Tokyo, Japan). The average BP of the last two measurements was used in the analyses. Hypertension was defined as either systolic BP (sBP) ≥140 mmHg, diastolic BP (dBP) ≥90 mmHg, or the use of antihypertensive medication. Fasting glucose, creatinine (Cre), and cystatin C (Cys C) were measured using COBAS 8000 (Roche Diagnostics).[15] Serum creatinine was measured using an enzymatic assay standardized to the isotope dilution mass spectroscopy method (CREA Plus, Roche Diagnostics, GmbH, Mannheim, Germany). Cystatin C was measured by a particle-enhanced turbidimetric immunoassay (Gentian, Moss, Norway). The inter-assay coefficient of variation for creatinine and cystatin C was 2.3 and 3.1%, respectively.[23] Due to the lack of an established international standard at baseline, the baseline cystatin C measurements were calibrated to the international reference ERM-DA471/IFCC (as previously described).[29] This standard was in use during data collection in RENIS-FU and RENIS-3. eGFR was based on Cre, Cys C or both using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[30,31] Three samples of first-void morning urine were collected on consecutive days, and fresh samples were analyzed for the urinary albumin to creatinine ratio (uACR mg/mmol).[20] All measurements and blood samples were collected in the morning between 08:00-10:00 a.m.

Statistical analyses

Descriptive statistics for normally distributed variables and skewed variables were given as the means (standard deviations: SD) and medians (IQR), respectively. Categorical variables were given as numbers (n) and percentages. Normally distributed proteins were modeled per SD increase in concentration, whereas proteins with skewed distributions were modeled per logarithmic unit increase after log2-transformation.

The association between proteins measured at baseline and a dichotomous variable for accelerated mGFR and eGFR decline was investigated using multiple logistic regression analyses. In studies of CKD, which include many patients with very rapid eGFR decline, accelerated GFR decline has often been defined as loss of GFR greater than 3.0 mL/min/1.73 m^2/year. To obtain a reasonable number of persons with accelerated GFR in this relatively healthy population, we instead defined it as the 10% steepest mGFR or eGFR decline slopes, calculated using linear mixed model for each GFR method adjusted for baseline age, sex, BMI, smoking, blood pressure medication, sBP and fasting glucose, using a within-person centered time-variable, as described in previous publications.[28,32,33] The slope obtained from a linear mixed model is considered to be more precise than a slope based on linear regression and a better surrogate marker of end-stage kidney disease, especially among those with GFR >60 ml/min/1.73 m^2.[34]

To test the differences between the odds ratios (OR) for the GFR methods in the logistic regressions (mGFR vs. each eGFR, or between eGFRcre and eGFRcys), we used the *suest* (seemingly unrelated estimation) command in STATA.

Incident CKD was defined as new-onset eGFR or mGFR <60 ml/min/1.73 m^2, a definition used by others.[21,35] Since the exact time of the event was not observed, the risk of incident CKD during the study period was assessed using interval-censored Cox regression analysis.[36] Censoring was performed at the first time interval incident CKD was assumed to have occurred. Thus, persons with prevalent CKD at baseline were categorized as left censored, i.e. the event occurred before the first

visit. Those who developed CKD in-between visits were categorized as interval censored, and those who had GFR >60 ml/min/1.73 m^2 at their last visit as right censored. To avoid misclassification if baseline GFR was < 60 ml/min/1.73m^2 whereas subsequent GFR was >60 ml/min/1.73m^2, we did not register this as CKD unless GFR fell below 60 ml/min/1.73m^2 at a later measurement. For each protein, the statistical significance of the differences between the hazard ratio (HR) for mGFR and the other three eGFR methods, were calculated with the bootstrap method from 1000 resamples with replacement of the participants in the study population.

Both the analyses of accelerated decline and incident CKD were adjusted for covariates in three different models: Unadjusted analysis (protein concentration only); adjustment for factors commonly included in biomarkers studies that may be related to creatinine and/or cystatin C production (non-GFR related factors influencing creatinine or cystatin C): age, sex, BMI, and tobacco smoking[19,37] (Model 1). In a Model 2 we also adjusted for the respective baseline measured or estimated GFR (Model 2), as doing so could reduce confounding of baseline eGFRcre or eGFRcys due to unmeasured non-GFR related factors. [15-17] The association between a protein biomarker and eGFR decline was judged to be dissimilar from the association with mGFR decline if the difference between the associations estimated as OR (Table 3) or HR (Table 4) was statistically significant with alpha set at 0.05 in any of these models.

All 1410 participants who attended the baseline examination and had one or more follow-up GFR measurements were included in this study. There were equal number of observations regardless of whether eGFR or mGFR was used. However, we excluded one participant with an extreme outlier in the Cys C measurement at RENIS-3, leaving 1409 participants included in the analysis. The protein biomarkers were measured at baseline only.

All statistical analyses were performed with STATA version 17.0 (StataCorp, College Station, TX). A p value of <0.05 was considered as statistically significant. Due to the number of multiple analyses with different outcomes, adjustment of the p values for multiple comparisons was considered. This has

been a controversial issue in epidemiological research.[38] We selected all proteins based on earlier studies with a high probability of being associated with the outcome of interest, thus having high pretest probability, which would not have been considered in commonly used methods for multiple comparisons adjustment. Accordingly, we decided not to adjust for multiple comparisons.

Results

Study characteristics

The baseline characteristics of the study cohort are shown in Table 2. There were only small differences between the subgroup with one or more follow-up examinations (n=1409; included in the current study) and the total RENIS baseline cohort (n=1627) (Table S3).

Accelerated GFR decline.

The mean (SD) mGFR decline rate was 1.07 ml/min/1.73m^2/year (0.5). The 140 persons who had an accelerated mGFR decline defined as the 10% with the steepest mGFR decline slope had a mGFR decline rate of ≥1.63 ml/min/1.73 m²/year. As reported in a previous publication from the RENIS study, the mean (SD) eGFRcre decline rate was similar to that of mGFR, but the mean eGFRcys decline rate was steeper, and the distribution (SD) wider, using cystatin C-based eGFR.[21] Using the eGFR equations with the 10% steepest eGFR decline slopes, the cutoffs for eGFRcre, eGFRcys and eGFRcyscre were ≥1.49 ml/min/1.73 m²/year, ≥2.64 ml/min/1.73 m²/year and ≥2.13 ml/min/1.73 m²/year, respectively.

In the unadjusted analysis, higher concentrations of four proteins were associated with accelerated mGFR decline, five with eGFRcre decline, six with eGFRcys and eGFRcyscre decline (Table 3). For four proteins, there were statistically significant differences in associations between eGFRcys decline and mGFR decline (TRAIL-R2, FABP4, TNFR2 showed higher OR and Umod lower OR for eGFRcys vs mGFR). No proteins showed significantly different associations between eGFRcre decline and mGFR decline in unadjusted analysis. For GAL-3 it was a statistically significant difference between mGFR

and eGFRcyscre. For three proteins, the associations with eGFR decline were statistically significant different when using eGFRcre versus eGFRcys (TNFR2, Tie2 and Umod).

Most associations and differences between the GFR methods remained after adjustment for age, sex, BMI and smoking (model 1), but many associations changed in the model that included baseline eGFR or mGFR, respectively (model 2). Only one out of four proteins for eGFRcre and one out of five proteins for eGFRcys in model 1 remained associated with eGFR decline after additional adjustment for baseline eGFR, while three out of three remained associated with mGFR decline after adjusting for baseline mGFR. Five and two proteins showed statistically significant different results between eGFR and mGFR in model 1 and model 2, respectively. (Model 2, Table 3).

Incident CKD

At baseline, 8 participants had CKD using mGFR, 95 developed CKD during follow-up, and the rest (n=1307) never developed CKD during the study period. For eGFRcre, eGFRcys and eGFRcyscre: 4, 5 and 3 had eGFR<60 ml/min/1.73 m^2/year at baseline, and 51, 96 and 62 developed CKD, respectively.

In the interval-censored Cox regression analysis using mGFR, eight proteins were associated with an increased risk of incident CKD in the unadjusted model, five with eGFRcre, eight with eGFRcys and seven with eGFRcyscre. In unadjusted analyses, there were statistically significant differences in the association of one protein for incident CKD using eGFRcre (suPAR), three for eGFRcys (MCP-1, TNFR2 and GDF-15), and one (Gal-3) for eGFRcyscre, compared to the results using mGFR. For three proteins (TNFR2, suPAR and Umod) there were also differences in the associations with incident CKD using eGFRcre vs. eGFRcys.

Most of the associations between proteins and incident CKD, and the different results compared to mGFR, remained similar after adjustment for age, sex, and BMI (Table 4, model 1). However, after additional adjustment for the corresponding measured or estimated baseline GFR most of the associations between baseline protein concentration and incident CKD changed. Four proteins remained associated with increased risk of incident CKD using mGFR (TRAIL-R2, TNFR2, GDF-15, and

MMP7). For eGFR, only two proteins remained associated with increased risk (GDF-15 with eGFRcys and MMP7 with all eGFRs). Only one protein (suPAR) remained statistically significantly differently associated with incident CKD compared to the association with incident CKD using mGFR (Table 4).

Discussion

In this cohort from the general population without diabetes, self-reported kidney disease or CVD at baseline, biomarkers showed divergent associations with the three eGFR methods compared to mGFR and among the eGFR methods themselves. Several of the differences between accelerated decline of mGFR and eGFR or between eGFRcre and eGFRcys were statistically significant in unadjusted models and when adjusting for age, sex and BMI. Two differences remained significant in the model that included baseline GFR. More biomarkers showed different associations with eGFRcys decline than with eGFRcre decline. Only MMP7 showed statistically significant associations with GFR decline and incident CKD using all GFR methods, regardless of adjustment.

These findings indicate the influence of non-GFR-related factors when assessing the association of proteins with eGFR decline. We have previously shown that eGFRcre and eGFRcys are influenced by inflammation, as assessed by TNFR2 and CRP, and by CKD risk factors.[14,15] The current results, showing a statistically significant difference between the GFR methods used and, e.g., associations with TNFR2, indicate that different associations can be found for some biomarkers when assessing GFR change using eGFR or mGFR. For example, muscle mass, obesity, and low-grade inflammation may affect the level of several biomarkers and at the same time influence the production rate of creatinine and/or cystatin C. Muscle wasting (reduced creatinine), increased body fat, and increased inflammation (increased cystatin C) during follow-up, which is commonly seen during aging, may lead to a stronger association with a decline in eGFRcys and a weaker association with the eGFRcre decline rate. Adjustment for factors that have been associated with non-GFR-related influence on creatinine and cystatin C (e.g., age, sex, obesity, and smoking in model 1) at baseline would be expected to reduce such influences. However, for most of the investigated proteins the differences

to mGFR persisted in model 1. Other nontraditional cardiovascular risk markers, such as fasting insulin levels, muscle mass and dimethylarginines, have also been found to influence creatinine and/or cystatin C levels along non-GFR-related pathways.[16] Since these risk factors are typically not available as covariates for adjustment in many studies, residual confounding may persist when using eGFR.

Several of the associations between proteins and eGFR decline, and the different associations compared to mGFR decline in the current study, were attenuated and no longer significant when adjusting for baseline eGFR or mGFR, respectively.[39] There may be several explanations for this, and one possibility is that the inclusion of baseline eGFR in the model partly blocks the non-GFR related effects on eGFR change, thereby also reducing the longitudinal confounding. Accordingly, adjusting for baseline GFR may adjust for some of the confounding factors so that change in eGFR is more reflective of true change in mGFR.

In line with the results of the present longitudinal study, several previous cross-sectional studies found cystatin C to be influenced by more non-GFR-related factors than creatinine.[14,16,17] We recently reported that eGFRcys overestimates GFR change rates compared to mGFR.[21] The distribution of the GFR decline rates was wider with eGFRcys than with mGFR, possibly due to the influence of non-GFR related factors.

Ten protein biomarkers in this study have previously been shown to be associated with eGFR in general population studies, of which nine were associated with eGFR decline.[3-13,27,40-42] Some of these biomarkers were not validated in separate cohorts, whereas others were validated but with mixed results. MMP7 have been associated with GFR decline in patients with diabetes using eGFRcre,[43,44] and was also associated with mGFR decline over a median of 5.6 years in the RENIS cohort.[28] Several of the proteins that have shown associations with eGFR decline in previous studies did not show any associations in our study, regardless of the method used to measure or estimate GFR. The different age distributions, population characteristics of the included persons in the studies, and follow-up

times, as well as different methods to measure proteins (ELISA, Luminex/proteomic) and statistical methods, could explain some of the diverging findings. Our cohort was relatively healthy at baseline, without self-reported CVD, kidney disease or diabetes, with mean estimated and measured baseline GFRs well within the normal range.[3-6,8,9] Thus, some of the observed GFR decline may represents age-related GFR decline rather than a pathological decline due to underlying disease. Although age-related GFR decline is an important driver of the high prevalence of CKD in the aging population, some proteins may be associated with specific disease processes in subgroups of people, which were not prevalent in the current study. However, our main objective was to validate associations found using eGFR with mGFR. We have no reason to believe that differences between methods will not hold in other more diseased populations. Similar non-GFR-related determinants of cystatin c and creatinine, such as inflammation, obesity, and CVD risk factors, have been found in different populations,[16,17,37] and their influence on eGFR may even be larger in patients than in healthy persons.

A study with elderly Swedish participants from two separate general population cohorts found associations between FABP4, suPAR, GDF-15, TRAIL-R2 and TNFR2 and annual eGFR decline and incident CKD.[3] In these Swedish cohorts, 74% and 40% of the participants had diabetes, which may explain the different results compared to the present study. However, among 20 proteins that were associated with eGFR decline in both cohorts, none were consistently associated with eGFR decline after adjustment for baseline eGFR. There may be several reasons for these findings, and it is controversial whether it is correct to adjust for the baseline value when investigating change rates of that same variable.[39,45] Nevertheless, our study shows that the results of eGFR decline is more similar to mGFR decline in models that adjust for the corresponding baseline GFR.

The results of this study need to be interpreted in the context of some strengths and limitations. The main strength is the longitudinal design with repeated GFR measurements by iohexol clearance during 11 years of follow-up. Additionally, we investigated the association with biomarkers that

represent different pathways likely involved in kidney function decline and CKD development and used different statistical methods to assess the relationship with different kidney outcomes using both mGFR and eGFR.

There are also limitations. Generalizability to other age groups and ethnicities is limited by only including North European study participants between 50-64 years. Misclassification of incident CKD could have occurred since censoring was done at the first visit with mGFR/eGFR <60 ml/min/1.73 m^2 without repeated eGFR/mGFR after three months. However, others have found similar risk patterns using this definition compared to CKD confirmed with later follow-up measurements, but with lower risk estimates.[35] The linear mixed model used to calculate the accelerated GFR decline outcome assumes a linear decline in GFR. Although this assumption may not hold for some participants, the non-linearity of the GFR trajectory in RENIS is modest and fairly similar for eGFR compared to mGFR.[21] The majority of proteins were measured using a Luminex assay with varying intra- and interassay CVs between 2.9–6.4% and 4.9–19.3% (four >10%), respectively. Thus, misclassification may have occurred. However, our mean intra- and interassay CVs were lower than or equal to those reported by others using the Luminex method. [3,46,47] Also, in a previous publication we explored the high inter-assay CV of MMP7, where one assay was found to have standards which deviated from the expected concentrations by 7.5–28%. By excluding the 30 participants on this assay, the inter-assay CV for the study population was reduced from 19.3% to 13.7%, and the association between the GFR outcomes and MMP7 even became slightly stronger.

To conclude, some associations between biomarkers and eGFR decline were different from the associations with mGFR decline. Thus, spurious associations with eGFR decline may be caused by the influence of non-GFR factors. Although the differences between the methods were attenuated after adjustment, particularly after adjustment for baseline eGFR, persisting differences for some biomarkers may be a problem in research on CKD pathophysiology and risk factors. The results of

studies using eGFR to identify biomarkers for GFR decline should therefore be interpreted with caution and preferably be validated with mGFR.

Authors' contribution

B.O.E and T.M were the principal investigators and designed the RENIS studies. D.S., T.M, I.T.E and B.O.E planned and carried out the analysis of the baseline serum protein biomarkers. I.T.E, T.M and B.O.E analyzed the data. I.T.E, N.B.R, T.M, B.O.E, J.V.N, M.D.S, and D.S drafted and revised the paper. All authors approved the final version of the manuscript.

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Disclosures

The authors have nothing to disclose.

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Data Sharing Statement

The data underlying this article cannot be shared publicly since this was not included in the research permission due to ethical considerations and the privacy of individuals who participated in the study. The data can be shared upon request as part of the research collaboration.

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Supplemental Table of Contents

Tables

Table 1: The 13 selected protein biomarkers analyzed with the Luminex multiplex method. Name of the protein, abbreviation, and function/involvement in pathways.

Abbreviation	Protein name	Size (kDa)	Function/pathways	Type of marker	Reference
MCP-1 (CCL2)	Monocyte Chemoattractant Protein 1	13	Inflammation, immunity.	Pro-fibrotic	[48,49]
TRAIL-R2	TNF-Related Apoptosis Inducing Ligand Receptor 2	48	Apoptosis, inflammation.	-	[50,51]
FABP4	Fatty Acid Binding Protein 4	15	Binds long-chain fatty acids, fat absorption, transportation, metabolism, inflammation, fibrosis.	Filtration and pro-fibrotic	[52-55]
TNFR2	Tumor Necrosis Factor Receptor 2	40	Inflammation, anti-apoptotic.	-	[56,57]
CD40Lig	CD40 receptor Ligand	18	Immunity.	Pro-fibrotic	[58,59]
GDF-15	Growth/Differentiation Factor 15	12 (dimer: 25-30)	Cell damage, inflammation, apoptosis.	Injury	[60]
Tie 2	TEK Tyrosine Kinase	75-78	Embryonic angiogenesis, immunity, anti-inflammatory.	-	[61]
MMP7	Matrix Metalloproteinase 7	19	Fibrosis, matrix remodulation, wound healing.	Pro-fibrotic, injury	[62,63]
suPAR	Soluble urokinase-type Plasminogen Activator Receptor	24 -66	Phosphate metabolism, apoptosis, inflammation.	-	[64,65]
MMP2	Matrix Metalloproteinase 2	72 (64 active)	Fibrosis, matrix remodulation. Neural system.	Pro-fibrotic	[66,67]
Umod	Uromodulin	85	Immunity.	Nephron mass	[68,69]
Gal-3	Galectin-3	30	Carbohydrate binding protein, apoptosis, immunity,	Pro-fibrotic	[70-72]

			antimicrobial, fibrosis.		
KIM-1	Kidney Injury Molecule 1	40-80	Kidney damage marker (proximal tubule), allergies, immunity.	Injury	[73]

Table 2: Baseline characteristics of the study cohort.

Baseline characteristics of RENIS participants with at least one follow-up GFR measurement	
Participants (n)	1409
Male sex (n)	696 (49%)
Age (years)	58.5 (54.5 - 61.4)
Height (cm)	170.7 (8.7)
Weight (kg)	79.6 (14.1)
BMI (kg/m²)	27.2 (3.9)
mGFR (ml/min/1.73 m²)	94.0 (14.3)
eGFRcre (ml/min/1.73 m²)	94.9 (9.4)
eGFRcys (ml/min/1.73 m²)	105.7 (12.1)
eGFRcyscre (ml/min/1.73 m²)	103.2 (11.2)
Urinary ACR (mg/mmol)[a]	0.2 (0.1 - 0.5)
Systolic BP (mmHg)	129 (17)
Diastolic BP (mmHg)	83 (10)
BP medication (n)	250 (18%)
RAS- inhibitors	143 (10%)
Fasting blood glucose (mmol/l)	5.3 (0.5)
LDL cholesterol (mmol/l)	3.7 (0.8)
HDL cholesterol (mmol/l)	1.5 (0.4)
Triglycerides (mmol/l)	1.2 (0.7)
Daily smoker (n)	949 (67%)
Current smoker (n)	278 (20%)
Previously smoker (n)	670 (48%)
Never smoker (n)	456 (32%)
Serum protein biomarkers:	
CD40Lig (ng/ml)	6.8 (2.1)
GDF-15 (ng/ml)	0.8 (0.4)
MCP-1 (ng/ml)	0.5 (0.4 - 0.6)
Tie2 (ng/ml)	16.2 (5.7)
TRAIL-R2 (ng/ml)	0.03 (0.03 - 0.04)
FABP4 (ng/ml)	9.5 (6.6 - 13.8)
MMP7 (ng/ml)	1.9 (0.80)
suPAR (ng/ml)	0.3 (0.2)
MMP2 (ng/ml)	315.3 (58.7)
Umod (ng/ml)	237.8 (96.0)
Gal-3 (ng/ml)	7.5 (1.9)
TNFR2 (ng/ml)	2.7 (0.6)

Abbreviations: RENIS: The Renal Iohexol Clearance Survey, BMI: body mass index, mGFR: measured glomerular filtration rate, eGFRcre/cys/cyscre: estimated GFR based on the CKD-EPI equation for creatinine, cystatin C or both, ACR: albumin to creatinine ratio, BP: blood pressure, RAS: renin-angiotensin system, LDL: low density lipoprotein, HDL: high density lipoprotein, CD40Lig: CD40 ligand receptor, GDF-15: growth/differentiation factor 15, MCP-1: monocyte

Mean (SD) for normally distributed variables, median (IQR) for skewed variables, and number and percentages (%) for

Variables with missing baseline values (n): smoke (5), TRAIL-R2 (1), FABP4 (3), TNFR2 (3), MMP7 (14) and suPAR (18).

[a] To convert ACR in mg/mmol to mg/g, multiply by 8.84.

Table 3: Odds ratios (OR) for accelerated mGFR and eGFR decline per doubling or standard deviation (SD) increase in baseline protein concentration.

Protein	Unadjusted		P-value for	Model 1		P-value for	Model 2		P-value for
	OR	95% CI	difference	OR	95% CI	difference	OR	95% CI	difference
MCP-1*									
mGFR	1.18	(0.87 to 1.59)		1.08	(0.76 to 1.52)		1.08	(0.77 to 1.52)	
eGFRcre	1.11	(0.80 to 1.55)	0.80	1.11	(0.79 to 1.57)	0.90	1.10	(0.78 to 1.56)	0.94
eGFRcys	1.31	(0.98 to 1.75)	0.61	1.28	(0.91 to 1.79)	0.99	1.00	(0.62 to 1.63)	0.80
eGFRcyscre	1.13	(0.82 to 1.55)	0.84	1.08	(0.76 to 1.53)	0.57	1.01	(0.70 to 1.46)	0.80
TRAIL-R2*									
mGFR	**1.35**	**(1.04 to 1.75)**		1.04	(0.73 to 1.47)		1.07	(0.75 to 1.53)	
eGFRcre	**1.53**	**(1.18 to 1.97)**	0.51	**1.41**	**(1.07 to 1.86)**	0.18	1.27	(0.94 to 1.71)	0.48
eGFRcys	**2.27**	**(1.50 to 3.44)**	**0.04**	**2.18**	**(1.49 to 3.18)**	**0.005**	1.33	(0.78 to 2.29)	0.51
eGFRcyscre	**1.52**	**(1.17 to 1.97)**	0.52	1.34	(0.99 to 1.83)	0.28	1.01	(0.63 to 1.63)	0.85
FABP4*									
mGFR	1.00	(0.85 to 1.19)		1.02	(0.81 to 1.29)		1.07	(0.84 to 1.37)	
eGFRcre	1.18	(0.94 to 1.48)	0.27	1.17	(0.86 to 1.60)	0.48	1.02	(0.76 to 1.35)	0.78
eGFRcys	**1.34**	**(1.08 to 1.66)**	**0.04**	**1.74**	**(1.25 to 2.44)**	**0.01**	1.01	(0.68 to 1.48)	0.79
eGFRcyscre	**1.32**	**(1.04 to 1.68)**	0.07	**1.44**	**(1.00 to 2.06)**	0.12	1.11	(0.79 to 1.55)	0.87
TNFR2									
mGFR	**1.40**	**(1.20 to 1.64)**		**1.21**	**(1.02 to 1.43)**		**1.28**	**(1.07 to 1.53)**	
eGFRcre	**1.24**	**(1.07 to 1.44)**	0.28[a1]	**1.19**	**(1.02 to 1.39)**	0.88[a2]	1.07	(0.91 to 1.26)	0.15
eGFRcys	**2.57**	**(2.07 to 3.18)**	**<0.001**	**2.45**	**(1.93 to 3.12)**	**<0.001**	1.10	(0.82 to 1.47)	0.38
eGFRcyscre	**1.44**	**(1.22 to 1.70)**	0.82	**1.31**	**(1.10 to 1.55)**	0.52	0.95	(0.77 to 1.17)	**0.03**
CD40Lig									
mGFR	1.03	(0.88 to 1.19)		1.00	(0.84 to 1.20)		1.00	(0.83 to 1.19)	
eGFRcre	1.10	(0.96 to 1.26)	0.49	1.07	(0.93 to 1.24)	0.56	1.09	(0.94 to 1.26)	0.45
eGFRcys	1.15	(0.98 to 1.35)	0.29	1.18	(1.00 to 1.39)	0.20	1.20	(0.96 to 1.50)	0.21
eGFRcyscre	1.11	(0.97 to 1.28)	0.44	1.10	(0.95 to 1.29)	0.42	1.09	(0.93 to 1.27)	0.48
GDF-15									
mGFR	1.29	(0.73 to 2.28)		**1.12**	**(1.00 to 1.25)**		**1.13**	**(1.01 to 1.26)**	
eGFRcre	1.10	(0.95 to 1.28)	0.60	1.06	(0.95 to 1.18)	0.54	1.01	(0.85 to 1.19)	0.28
eGFRcys	1.69	(0.96 to 2.96)	0.51	1.38	(0.65 to 2.94)	0.58	1.13	(0.99 to 1.29)	0.95
eGFRcyscre	1.19	(0.83 to 1.71)	0.81	1.11	(1.00 to 1.25)	1.00	0.96	(0.75 to 1.24)	0.28
Tie2									
mGFR	1.02	(0.87 to 1.20)		0.87	(0.73 to 1.03)		0.87	(0.74 to 1.04)	
eGFRcre	0.89	(0.74 to 1.08)	0.29[a3]	0.90	(0.74 to 1.09)	0.79	0.87	(0.71 to 1.06)	0.97
eGFRcys	**1.29**	**(1.09 to 1.52)**	0.05	1.14	(0.95 to 1.35)	**0.03**	1.12	(0.89 to 1.41)	0.08
eGFRcyscre	1.04	(0.88 to 1.23)	0.86	0.99	(0.83 to 1.17)	0.31	0.94	(0.78 to 1.12)	0.59
MMP7									
mGFR	**1.80**	**(1.53 to 2.13)**		**1.65**	**(1.38 to 1.97)**		**1.74**	**(1.44 to 2.09)**	
eGFRcre	**1.54**	**(1.31 to 1.81)**	0.19	**1.46**	**(1.23 to 1.72)**	0.31	**1.34**	**(1.13 to 1.59)**	**0.04**
eGFRcys	**1.90**	**(1.62 to 2.24)**	0.64	**1.86**	**(1.57 to 2.20)**	0.35	**1.75**	**(1.37 to 2.23)**	0.98
eGFRcyscre	**1.83**	**(1.56 to 2.15)**	0.89	**1.71**	**(1.45 to 2.02)**	0.79	**1.52**	**(1.27 to 1.81)**	0.08
suPAR									
mGFR	1.05	(0.93 to 1.18)		0.97	(0.82 to 1.16)		0.98	(0.82 to 1.17)	
eGFRcre	1.05	(0.94 to 1.18)	0.95	1.02	(0.90 to 1.15)	0.69	1.00	(0.86 to 1.16)	0.86
eGFRcys	1.14	(0.98 to 1.31)	0.40	1.13	(1.00 to 1.27)	0.20	1.04	(0.85 to 1.27)	0.64
eGFRcyscre	1.11	(0.98 to 1.26)	0.53	1.07	(0.95 to 1.20)	0.40	1.02	(0.88 to 1.18)	0.72
MMP2									
mGFR	0.94	(0.79 to 1.12)		0.97	(0.82 to 1.17)		0.97	(0.82 to 1.16)	
eGFRcre	1.03	(0.88 to 1.21)	0.46	1.02	(0.88 to 1.19)	0.67	0.99	(0.84 to 1.17)	0.89
eGFRcys	0.98	(0.82 to 1.17)	0.78	0.95	(0.79 to 1.14)	0.84	0.84	(0.66 to 1.07)	0.33
eGFRcyscre	1.03	(0.88 to 1.20)	0.46	1.01	(0.87 to 1.18)	0.75	1.00	(0.85 to 1.17)	0.85
Umod									
mGFR	**0.76**	**(0.64 to 0.90)**		0.94	(0.78 to 1.14)		0.92	(0.76 to 1.12)	
eGFRcre	**0.81**	**(0.67 to 0.98)**	0.62[a4]	**0.79**	**(0.65 to 0.97)**	0.23	0.84	(0.69 to 1.03)	0.52
eGFRcys	**0.52**	**(0.43 to 0.63)**	**0.003**	**0.61**	**(0.49 to 0.75)**	**0.002**	0.85	(0.62 to 1.16)	0.64
eGFRcyscre	**0.72**	**(0.60 to 0.87)**	0.67	**0.77**	**(0.63 to 0.94)**	0.16	0.89	(0.71 to 1.11)	0.80
Gal-3									
mGFR	0.98	(0.83 to 1.15)		1.07	(0.89 to 1.30)		1.09	(0.91 to 1.21)	
eGFRcre	**1.19**	**(1.04 to 1.37)**	0.07	1.18	(1.02 to 1.36)	0.46	1.11	(0.95 to 1.30)	0.87
eGFRcys	1.10	(0.96 to 1.26)	0.27	1.25	(1.06 to 1.47)	0.24	0.97	(0.76 to 1.24)	0.45
eGFRcyscre	**1.21**	**(1.06 to 1.38)**	**0.04**	1.25	(1.08 to 1.45)	0.22	1.16	(0.99 to 1.36)	0.62

Accelerated GFR decline defined as the 10% with the steepest annual GFR decline slope for the corresponding GFR method.

Abbreviations: mGFR: measured glomerular filtration rate, eGFR: estimated GFR, cre: creatinine, cys: cystatin C, MCP-1: monocyte chemoattractant protein-1, TRAIL-R2: TNF-related apoptosis-inducing ligand receptor 2, FABP4: fatty acid binding protein 4, TNFR-2: tumor necrosis factor receptor 2, CD40Lig: CD40 ligand receptor, GDF-15: growth/differentiation factor 15, Tie2: TEK tyrosine Kinase, MMP7: matrix metalloproteinase 7, suPAR: soluble urokinase-type plasminogen activator receptor, MMP2: matrix metalloproteinase 2, Umod: uromodulin, Gal-3: galectin-3.

Model 1: Sex, age, body mass index (BMI), smoke (now, previously, newer).

Model 2: Model 1 + baseline GFR.

P-value for statistically significant differences between mGFR and the respective eGFR from creatinine, cystatine C or both.

[a] A statistically significant difference between eGFRcre and eGFRcys:

[a1]P-value: <0.001, [a2]P-value: <0.001, [a3]P-value:0.004, [a4]P-value: 0.001.

*Log2 transformed.

Table 4: Hazard ratios (HR) for incident CKD per doubling or standard deviation (SD) increase in baseline protein concentration.

Protein	Unadjusted		P-value for	Model 1		P-value for	Model 2		P-value for
	HR	95% CI	difference	HR	95% CI	difference	HR	95% CI	difference
MCP-1*									
mGFR	0.89	(0.58 to 1.37)		0.87	(0.55 to 1.37)		0.81	(0.52 to 1.27)	
eGFRcre	1.00	(0.58 to 1.73)	0.55	0.95	(0.53 to 1.70)	0.67	0.93	(0.54 to 1.59)	0.76
eGFRcys	1.24	(0.86 to 1.78)	**0.04**	1.17	(0.78 to 1.75)	0.19	1.06	(0.74 to 1.51)	0.43
eGFRcyscre	1.06	(0.64 to 1.75)	0.40	1.00	(0.59 to 1.68)	0.56	0.87	(0.51 to 1.46)	0.91
TRAIL-R2*									
mGFR	**1.64**	**(1.37 to 1.96)**		**1.54**	**(1.27 to 1.87)**		**1.48**	**(1.18 to 1.87)**	
eGFRcre	**1.67**	**(1.20 to 2.31)**	0.87	**1.58**	**(1.08 to 2.31)**	0.78	1.37	(0.81 to 2.30)	0.57
eGFRcys	**1.62**	**(1.44 to 1.83)**	0.98	**1.44**	**(1.25 to 1.67)**	0.71	1.00	(0.63 to 1.58)	0.09
eGFRcyscre	**1.57**	**(1.26 to 1.96)**	0.69	**1.39**	**(1.08 to 1.80)**	0.50	0.82	(0.46 to 1.44)	0.05
FABP4*									
mGFR	**2.00**	**(1.58 to 2.53)**		**1.69**	**(1.28 to 2.23)**		1.10	(0.82 to 1.48)	
eGFRcre	**1.91**	**(1.39 to 2.64)**	0.84	**1.86**	**(1.27 to 2.73)**	0.98	1.19	(0.77 to 1.83)	0.72
eGFRcys	**2.37**	**(1.87 to 3.01)**	0.16	**1.70**	**(1.28 to 2.26)**	0.51	0.98	(0.72 to 1.34)	0.36
eGFRcyscre	**2.31**	**(1.75 to 3.06)**	0.31	**1.89**	**(1.37 to 2.61)**	0.63	0.99	(0.67 to 1.46)	0.54
TNFR2									
mGFR	**1.47**	**(1.37 to 1.57)**		**1.33**	**(1.25 to 1.42)**		**1.15**	**(1.05 to 1.26)**	
eGFRcre	**1.37**	**(1.20 to 1.57)**	0.36 [a1]	**1.27**	**(1.12 to 1.44)**	0.52[a2]	1.09	(0.88 to 1.36)	0.64
eGFRcys	**1.69**	**(1.63 to 1.75)**	**<0.001**	**1.63**	**(1.55 to 1.72)**	**<0.001**	1.01	(0.85 to 1.21)	0.40
eGFRcyscre	**1.58**	**(1.47 to 1.71)**	0.08	**1.46**	**(1.34 to 1.58)**	0.13	0.95	(0.80 to 1.14)	0.16
CD40Lig									
mGFR	1.08	(0.91 to 1.29)		1.05	(0.89 to 1.24)		1.04	(0.89 to 1.23)	
eGFRcre	0.97	(0.72 to 1.32)	0.31	0.95	(0.70 to 1.29)	0.36	0.93	(0.96 to 1.27)	0.43
eGFRcys	1.08	(0.89 to 1.31)	0.93	0.99	(0.86 to 1.15)	0.46	0.91	(0.77 to 1.08)	0.17
eGFRcyscre	1.04	(0.82 to 1.32)	0.61	0.97	(0.78 to 1.20)	0.31	0.96	(0.75 to 1.22)	0.34
GDF-15*									
mGFR	**1.67**	**(1.26 to 2.21)**		**1.55**	**(1.11 to 2.15)**		1.25	(0.90 to 1.74)	
eGFRcre	1.27	(0.91 to 1.76)	0.35	1.09	(0.72 to 1.64)	0.31[a3]	0.76	(0.48 to 1.20)	0.09
eGFRcys	**2.18**	**(1.72 to 2.77)**	**0.04**	**2.18**	**(1.59 to 2.99)**	0.05	1.13	(0.76 to 1.68)	0.67
eGFRcyscre	**2.07**	**(1.47 to 2.91)**	0.19	**1.83**	**(1.23 to 2.73)**	0.36	0.99	(0.60 to 1.62)	0.31
Tie2									
mGFR	1.03	(0.85 to 1.25)		1.06	(0.87 to 1.29)		0.95	(0.77 to 1.17)	
eGFRcre	1.12	(0.87 to 1.46)	0.50	1.14	(0.85 to 1.53)	0.56	1.08	(0.82 to 1.42)	0.49
eGFRcys	1.11	(0.93 to 1.33)	0.45	1.05	(0.88 to 1.27)	0.97	1.12	(0.92 to 1.36)	0.33
eGFRcyscre	1.09	(0.87 to 1.37)	0.59	1.09	(0.86 to 1.40)	0.73	1.00	(0.79 to 1.26)	0.81
MMP7									
mGFR	**1.85**	**(1.62 to 2.11)**		**1.75**	**(1.53 to 1.99)**		**1.53**	**(1.34 to 1.75)**	
eGFRcre	**1.71**	**(1.47 to 1.99)**	0.48	**1.65**	**(1.39 to 1.96)**	0.61	**1.42**	**(1.13 to 1.79)**	0.53
eGFRcys	**1.88**	**(1.69 to 2.09)**	0.81	**1.80**	**(1.58 to 2.06)**	0.71	**1.65**	**(1.35 to 2.02)**	0.48
eGFRcyscre	**1.89**	**(1.67 to 2.14)**	0.81	**1.75**	**(1.51 to 2.03)**	0.98	**1.48**	**(1.23 to 1.80)**	0.74
suPAR									
mGFR	**1.11**	**(1.06 to 1.15)**		1.08	(0.94 to 1.24)		1.11	(0.98 to 1.25)	
eGFRcre	0.95	(0.67 to 1.35)	**0.04[a4]**	0.81	(0.54 to 1.23)	**0.04[a5]**	**0.61**	**(0.41 to 0.90)**	**0.01[a6]**
eGFRcys	**1.14**	**(1.10 to 1.17)**	0.14	**1.13**	**(1.04 to 1.22)**	0.30	1.08	(0.90 to 1.30)	0.71
eGFRcyscre	**1.11**	**(1.04 to 1.19)**	0.81	1.06	(0.85 to 1.33)	0.77	0.81	(0.61 to 1.10)	0.08
MMP2									
mGFR	1.12	(0.93 to 1.35)		1.04	(0.87 to 1.24)		1.08	(0.91 to 1.30)	
eGFRcre	1.27	(1.02 to 1.60)	0.09	1.19	(0.95 to 1.48)	0.06	1.20	(0.88 to 1.64)	0.27
eGFRcys	1.18	(1.01 to 1.38)	0.47	1.12	(0.98 to 1.27)	0.29	**1.24**	**(1.03 to 1.49)**	0.24
eGFRcyscre	1.18	(0.93 to 1.48)	0.50	1.09	(0.89 to 1.35)	0.43	1.01	(0.81 to 1.27)	0.71
Umod									
mGFR	**0.74**	**(0.58 to 0.94)**		**0.72**	**(0.56 to 0.93)**		0.82	(0.64 to 1.06)	
eGFRcre	0.91	(0.66 to 1.26)	0.09[a7]	0.92	(0.65 to 1.30)	0.08[a8]	1.13	(0.84 to 1.52)	0.07
eGFRcys	**0.67**	**(0.50 to 0.89)**	0.30	**0.71**	**(0.53 to 0.95)**	0.81	0.98	(0.70 to 1.37)	0.26
eGFRcyscre	0.74	(0.53 to 1.05)	0.94	0.75	(0.53 to 1.07)	0.81	1.04	(0.73 to 1.48)	0.20
Gal-3									
mGFR	**1.25**	**(1.07 to 1.45)**		1.14	(0.97 to 1.35)		1.09	(0.90 to 1.31)	
eGFRcre	**1.38**	**(1.15 to 1.64)**	0.06	**1.32**	**(1.09 to 1.60)**	**0.03**	1.18	(0.98 to 1.43)	0.36
eGFRcys	**1.30**	**(1.13 to 1.50)**	0.34	**1.18**	**(1.01 to 1.38)**	0.64	1.09	(0.97 to 1.22)	0.95
eGFRcyscre	**1.38**	**(1.18 to 1.63)**	**0.02**	**1.28**	**(1.07 to 1.52)**	0.06	1.12	(0.96 to 1.32)	0.61

Incident CKD is defined as new-onset GFR <60 ml/min/1.73m^2 during follow-up.

A total of 1409 individuals are included in the analysis, the respective numbers of baseline CKD and incident CKD for the GFR methods are: mGFR; n=8 and, n=95. eGFRcre; n=4 and n=51. eGFRcys; n=5 and n=96. eGFRcyscr; n=3 and n=62.

Abbreviations: mGFR: measured glomerular filtration rate, eGFR: estimated GFR, cre: creatinine, cys: cystatin C, MCP-1: monocyte chemoattractant protein-1, TRAIL-R2: TNF-related apoptosis-inducing ligand receptor 2, FABP4: fatty acid binding protein 4, TNFR-2: tumor necrosis factor receptor 2, CD40Lig: CD40 ligand receptor, GDF-15: growth/differentiation factor 15, Tie2: TEK tyrosine Kinase, MMP7: matrix metalloproteinase 7, suPAR: soluble

Model 1: Sex, age, body mass index (BMI), smoke (now, previously, newer).

Model 2: Model 1 + baseline GFR.

Difference between eGFrcre and eGFRcys: [a1] P-value: <0.001. [a2] P-value: <0.001. [a3] P-value: 0.04. [a4] P-value: 0.004. [a5] P-value: 0.002. [a6] P-value: 0.004. [a7] P-value: 0.008. [a8] P-value: 0.04.

* Log2 transformed.

Figures and Figure Legends

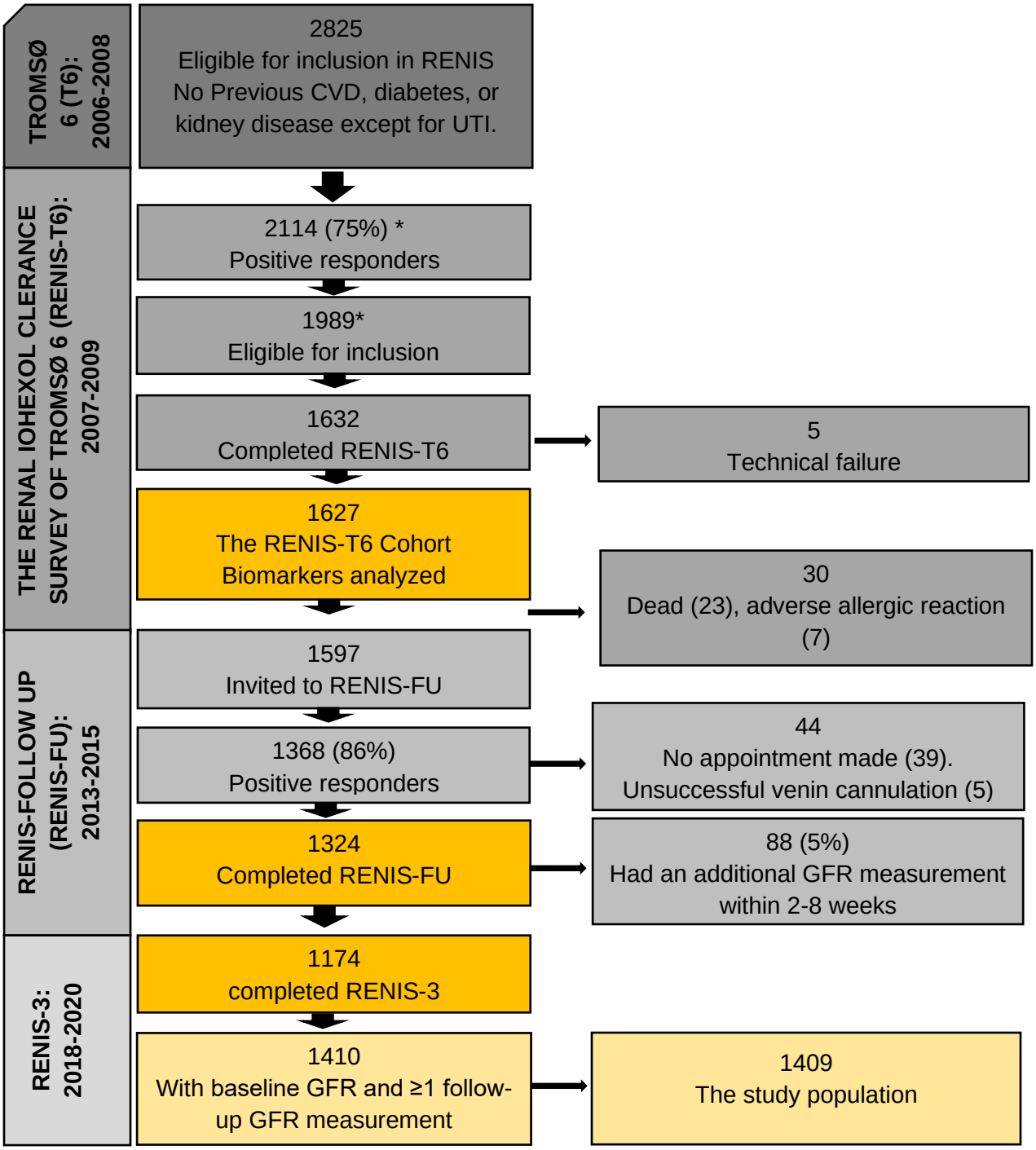

Figure 1: An overview of the Renal Iohexol Clearance Survey with number (n) of participants included and response rate (%) with relevant remarks.

*Miscount in previous publications (Earlier numbers are 2107 and 1982, the correct numbers are 2114 and 1989, respectively).

Abbreviation: CVD: Cardiovascular disease, UTI: urinary tract infection, GFR: Glomerular filtration rate.

Supplemental Material

(Separate document)