

Full Title: GFR decline predicts total mortality and mediates the effect of tryptophan metabolism on death risk in type 2 diabetes

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Abstract

Context: The independent role of glomerular filtration rate (GFR) decline in shaping the risk of mortality in people with type 2 diabetes has only been partially addressed.

Objective: The objective of the study was twofold: i) to investigate the association between all-cause mortality and eGFR changes over time; ii) to understand whether renal dysfunction mediates the effect of tryptophan metabolism on death risk.

Design: Prospective study with an average follow-up of 14.8 years.

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1 Setting: Research Hospital.

2 Patients: The aggregate Gargano Mortality Study included 962 patients with type 2 diabetes who
3 had at least three eGFR recordings and at least 1.5 years of follow-up.

4 Interventions: This was an observational study, with no intervention.

5 Main Outcome Measures: Rate of all-cause mortality

6 Results: Age and sex adjusted annual incident rate of mortality was 2.75 events per 100 person-
7 years. The median annual rate of decline of eGFR was 1.3 ml/min per 1.73 m² per year (range -
8 3.7;7.8). The decline of kidney function was strongly and independently associated with the risk
9 of death. Serum kynurenine-to-tryptophan ratio (KTR) was associated with both eGFR decline and
10 all-cause mortality. Causal mediation analysis showed that 24.3% of the association between KTR
11 and mortality was mediated by eGFR decline.

12 Conclusions: In patients with type 2 diabetes, eGFR decline is independently associated with the
13 risk of all-cause mortality and mediates a significant proportion of the association between
14 tryptophan metabolism and death.

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17 Diabetes is recognised as the fastest growing chronic disease in the world (1) associated with
18 substantial increase of mortality. Approximately 6.7 million adults are estimated to have died from
19 diabetes or its complications in 2021 (1), making diabetes a leading cause of death worldwide (2).
20 The rate of mortality in the general population is decreased significantly in recent years thanks to
21 interventions that reduce the risk of myocardial infarction, such as lipid-lowering and
22 antihypertensive therapy, and better glycaemic control over time (3-5). However, the risk of death

in patients with type 2 diabetes is still high, being about double that of people without the disease (6). Several studies in high-risk people have shown a strong relationship between the annual rate of decline in estimated glomerular filtration rate (eGFR) and all-cause mortality (7-11). Unfortunately, such relationship has been only sparsely addressed in people with type 2 diabetes (12,13). Therefore, the main aim of our study was to investigate the association between changes in renal function over a long follow-up period and the risk of all-cause mortality in people with type 2 diabetes from Southern Italy. Furthermore, since serum kynurenine-to-tryptophan ratio (KTR) is associated with both the decline of eGFR and the rate of mortality (14-16) we also investigated whether kidney dysfunction plays the role of mediator between tryptophan metabolism and death risk in type 2 diabetes.

Materials and Methods

Study population

The aggregate Gargano Mortality Study (aGMS) consists of 2,140 people with type 2 diabetes recruited according to the same design and procedures from 2000 to 2008 (first period) and from 2008 to 2011 (second period) at the Endocrinology Unit of the IRCCS (Istituto di Ricovero e Cura a Carattere Scientifico) “Casa Sollievo della Sofferenza”, San Giovanni Rotondo and followed until February 2023. The only inclusion criterion was the presence of type 2 diabetes according to the 2003 American Diabetes Association criteria. Exclusion criteria were dialysis or transplantation and poor life expectancy for non-diabetes-related diseases. Any analysis using data from aGMS data is adjusted for the “recruitment period” (see below). This specific study was carried out in those 962 patients who had at least three eGFR recordings and at least 1.5 years of follow-up. The study and the informed consent procedures were approved by the local Institutional

Ethic Committee IRCCS “Casa Sollievo della Sofferenza”. All participants gave written consent.

The study was conducted and reported according to the STROBE checklist (17).

Exposure

In the present study, the exposure was the rate of eGFR decline over the time. We measured standardized serum creatinine using the modified kinetic Jaffè reaction (Hitachi 737 Autoanalyzer), calibrated to be traceable to an isotope dilution mass spectrometry (IDMS). GFR was estimated for each patient using the Chronic Kidney Disease Epidemiology Collaboration formula (18) derived from serum creatinine values at baseline and during follow-up. Repeated-measures, longitudinal, multilevel (mixed-effects) models with follow-up time as a fixed effect were used to compute the annual change in eGFR (measured as mL/min per 1.73 m²) for each participant.

Study endpoint

The vital status of all participants was verified by interrogating the Italian Health Card Database upon data anonymization (<https://sistemats1.sanita.finanze.it/wps/portal/>).

Clinical and biochemical measurements

Duration of type 2 diabetes and current glucose-, blood pressure- and lipid-lowering treatments were recorded for all patients. A physical examination, including measurement of height, weight and blood pressure, was performed on all subjects enrolled in the study. A fasting venous blood sample was taken from all patients for the measurement of serum total cholesterol, HDL cholesterol, triglycerides, glycated hemoglobin (HbA1c) and creatinine.

1 Urinary albumin and creatinine concentration were determined by the nephelometric method
2 (Behring Nephelometer Analyzer; Behring, Marburg, Germany) and the Jaffè reaction rate
3 method, respectively and were determined on an early morning first void sterile urine sample.
4 Urinary albumin excretion was calculated and reported as urinary albumin-to-creatinine ratio
5 (ACR). Increased UAE was diagnosed as microalbuminuria if urinary albumin-to-creatinine ratio
6 was > 2.5 mg/mmol in men and > 3.5 mg/mmol in women and ≤ 30 mg/mmol in both gender and
7 macroalbuminuria if albumin-to-creatinine ratio was > 30 mg/mmol in both gender.

8 Tryptophan, kynurenine and their ratio KTR were quantified using baseline fasting serum samples
9 and the AbsoluteIDQ™ p180 Kit (BIOCRATES Life Sciences), as previously described
10 (14,16,19).

11 Briefly, sample handling was performed by a Hamilton Microlab START™ robot (Hamilton
12 Bonaduz AG, Bonaduz, Switzerland) and a Ultravap nitrogen evaporator (Porvair Sciences,
13 Leatherhead, U.K.), beside standard laboratory equipment. Mass spectrometric analyses were done
14 on an API 4000 triple quadrupole system (SCIEX Deutschland GmbH, Darmstadt, Germany)
15 equipped with a 1260 Series HPLC (Agilent Technologies Deutschland GmbH, Böblingen,
16 Germany) and a HTC-xc PAL auto sampler (CTC Analytics, Zwingen, Switzerland) controlled by
17 the software Analyst 1.6.2. For the LC-part, compounds were identified and quantified based on
18 scheduled multiple reaction monitoring measurements (sMRM), for the FIA-part on MRM. Data
19 evaluation for quantification of metabolite concentrations and quality assessment was performed
20 with the software MultiQuant 3.0.1 (Sciex) and the MetIDQ™ software package. Metabolite
21 concentrations were calculated using internal standards and reported in μM . KTR was calculated
22 through the MetIDQ RatioExplorer Module.

1 Statistical analysis

2 Patients' baseline characteristics were reported as mean $\pm$ standard deviation (SD) or median
3 (range) and frequency (percentage) for continuous and categorical variables, respectively. Possible
4 differences in case-mix across sub-samples used for different analyses were investigated
5 measuring the Standardized Mean Differences (SMD). Missing data rates in subjects' covariates
6 at baseline used in this study (i.e., gender, age, BMI, duration of disease, HbA1c, baseline eGFR,
7 and medications) were, when present, always less than 5%. Missing data, therefore, were imputed
8 using a random forest approach (20). Age and sex-adjusted mortality rate was estimated using
9 Poisson models and was reported as number of deaths per 100 person-years. The overall survival
10 was defined as the time between enrollment and death; for subjects who did not experience the
11 end point, survival time was censored at the time of the last available follow-up visit. Time-to-
12 death analyses were performed using univariable and multivariable Cox proportional hazards
13 regression models and risks were reported as hazard ratios (HR) along with their 95% confidence
14 intervals (CI). To properly assess the most reliable relationship between the rate of eGFR decline
15 and all-cause mortality rate, eGFR decline was included into Cox models as: 1) a linear term only,
16 2) a quadratic term only, 3) both linear and quadratic terms. Goodness of fit of each Cox model
17 was evaluated by Akaike Information Criterion (AIC). The model including the linear term only
18 achieved the best fit (i.e., minimum AIC), a finding that is fully compatible with the linear
19 relationship observed between eGFR decline and mortality rate. The representation of eGFR
20 decline tertiles was also provided, for ease of clinical interpretation. Two separate Cox models
21 were performed. The first model was adjusted only for the recruitment period (0 or 1); the second
22 model included also demographics characteristics (i.e., sex and age at recruitment), diabetes-

related parameters (i.e., basal body mass index, HbA1c, eGFR and disease duration) and medications (i.e., glucose-, blood pressure- and lipid-lowering treatments at recruitment).

To investigate whether the eGFR annual decline acts as a mediator of the relationship between KTR and mortality risk, a causal mediation analysis was performed (21). The KTR values were first logarithmized and then standardized. Prior to assess causal mediation analysis, we checked if: i) KTR was associated with all-cause mortality using a fully adjusted Weibull regression model; ii) eGFR annual decline and KTR were correlated. Then, a causal mediation analysis was conducted using the 'mediate' function defined in the 'mediation' package in R. This function provided non-parametric estimates of the total, direct, and indirect effects, based on 1000 bootstrap replication. A p-value < 0.05 was considered for statistical significance. Statistical analyses and graphs were performed using R software.

Results

Patient characteristics

The main clinical features of the 962 study individuals are summarized in Table 1. To provide some clues as to how the subsets used in this study are representative of the entire cohort, the clinical characteristics of all 2140 aGMS individuals and the 575 used for metabolomic studies are also shown in the same Table 1 (indeed, SMD ranged 0.029-0.173, thus suggesting that differences across the three samples were negligible). Overall, the mean age of patients was 60.8 ± 9.7 years, 47.3% were women, mean duration of diabetes was 10.8 ± 9.0 years and the mean value of HbA1c was $8.5 \pm 1.9\%$. A total of 600 (62.4%) and 419 (43.6%) patients were on blood pressure- and lipid-lowering treatment, respectively. Mean eGFR value was 79.2 ± 21.5 mL/min/1.73 m². During

a median follow-up of 14.8 ± 5.5 years, 452 (47%) patients died. Age and sex adjusted annual incident rate of mortality was 2.75 events per 100 person-years. The median annual rate of eGFR decline was 1.3 ml/min per 1.73 m^2 per year (range -3.7-7.8) (Table 1).

Association between eGFR decline and all-cause mortality in the whole population

A strong association was observed between the annual rate of eGFR decline and mortality: for each ml/min increase in the annual rate of eGFR loss, there was a 31% increase in all-cause mortality, independent of all possible confounders we were able to take into account, including gender, age, BMI, duration of disease, HbA1c, baseline eGFR, glucose-, blood pressure- and lipid-lowering treatments [adjusted HR 1.31 (1.21-1.41), $p < 0.001$] (Table 2). To facilitate clinical interpretation, patients were sub-grouped according to tertiles of eGFR annual decline. Clinical features and incidence rate of all-cause mortality across eGFR tertiles are shown in Supplementary Table 1 (22). As compared to those with less pronounced eGFR decline (tertile 1), patients with the most rapid decline (tertile 3) had significantly higher age, BMI, disease duration, triglycerides, systolic blood pressure, ACR, glycated hemoglobin values and higher prevalence of blood pressure- and lipid-lowering treatments. They also had significantly lower baseline eGFR values. In the fully adjusted model, patients in tertile 2 and tertile 3 had 45% and 81% higher mortality rate than those in tertile 1 (Table 3, Figure 1).

Association between eGFR decline and all-cause mortality in individuals with information on albuminuria

Of note, in the 637 representative individuals for whom information on albuminuria was available (clinical features shown in Supplementary Table 2) (22), the association between eGFR annual

decline and all-cause mortality (HR 1.31, 95%CI 1.21-1.41, $p < 0.001$) did not change when also ACR was considered in the fully adjusted model (HR= 1.33, 95%CI = 1.21-1.46, $p < 0.001$).

Subgroup analyses

In subgroup analyses, the association between eGFR decline and mortality, was significantly stronger in individuals relatively younger and with better glycaemic control (individual age and HbA1c below the median value of the entire cohort; p for interaction = 0.01 and 0.02, respectively, Figure 2).

Mediation analysis explaining association between KTR and all-cause mortality

In the subset of 575 representative patients for whom KTR data were available (clinical features described in Table 1) we found a significant association between KTR and both rate of eGFR decline ($R^2=0.16$, $p=0.0002$) and all-cause mortality (fully adjusted HR per 1 SD increase in KTR values=1.33, 95%CI=1.09-1.63, $p=0.005$). In this subset, the adjusted association between eGFR annual decline and all-cause mortality (HR 1.29, 95%CI 1.17-1.43) was slightly attenuated when also KTR was considered in the fully adjusted model (HR= 1.27, 95%CI = 1.14-1.40). Indeed, causal mediation analysis showed that a significant and non-trivial proportion of the association between KTR and all-cause mortality was mediated by eGFR decline (i.e., 24.3%, $p=0.006$).

Discussion

In this longitudinal study including 962 patients with type 2 diabetes we observed a direct, independent relationship between the annual decline in kidney function and the rate of all-cause mortality. This association is clinically significant, with each milliliter of eGFR decline increasing

1 the risk of death by 31%. In addition, when looking at tertiles of eGFR decline, individuals with
2 the fastest loss of renal function (i.e., belonging to tertile 3) showed an 81% increased risk of
3 mortality compared to individuals of tertile 1. Of note, the association between eGFR decline and
4 mortality was modified by both age and HbA1c, being significantly stronger in relatively younger
5 subjects and in those with better glycaemic control meaning that the deleterious effect of kidney
6 dysfunction on the risk of death is more evident when other risk factors such as age and poor
7 glycaemic control are less prevalent. Finally, causal mediation analysis showed that eGFR decline
8 mediates approximately one fourth of the association between mortality and KTR, a marker of
9 tryptophan downstream metabolism that recognizes two alternative pathways: one that through
10 kynurenine is proinflammatory and another that synthesizes 5 methoxy-tryptophan, a molecule
11 with anti-inflammatory properties (23). Tryptophan depletion has been reported to be associated
12 with impaired kidney function (24). The degradation of tryptophan in the kynurenine pathway is
13 mediated by the enzyme indoleamine 2,3-dioxygenase (IDO) of which KTR is a proxy reflecting
14 its activity. It is of note that in the general population the KTR was associated with incident CKD
15 and GFR decline (25). This latter association has been reported also in patients with type 2 diabetes
16 by us and others (14,15) which makes possible to hypothesize a deleterious effect of kynurenine
17 and kynurenine metabolites on mesangial cells proliferation (26) and kidney fibrosis (27) as well
18 as ROS production, cell damage and apoptosis in the kidney (28).

19 Recently, several studies have reported a positive association between the annual rate of decline
20 in eGFR and all-cause mortality in both the general population and in people with chronic kidney
21 disease (7-11). Similar data have also been reported twice in people with diabetes, although a long
22 follow-up like ours was available only in Chinese (12) but not in people of different ancestral
23 origin, mostly Europeans (13). In addition, eGFR decline is positively associated with major

adverse cardiovascular events, including mortality, in people with type 2 diabetes from France (29). Therefore, taken altogether our and previous (12,13,29) studies clearly indicate that the association between eGFR decline and mortality rate is a generalizable phenomenon independent of ethnicity and environmental background. It is worth noting that novel antidiabetic drugs such as SGLT2 inhibitors and GLP1-RA, which are able to slow GFR decline, also reduce all-cause mortality, thus reinforcing the plausibility of a causal role of rapid GFR decline in shaping survival probability in people with type 2 diabetes (30). The mechanisms underlying the association between rapid decline in eGFR and increased risk of all-cause mortality are beyond the scope of this study, but several mechanisms could be hypothesized. First, patients with worsening renal function have an atherogenic risk profile that predisposes to cardiovascular events and heart failure (7,31), both major causes of death in people with diabetes. The relationship between GFR decline and heart failure is also supported by the common effect of SGLT2 inhibitors on both kidney function decline and heart failure, suggesting common etiological factors between the two outcomes.

This may well be due to endothelial dysfunction, oxidative stress and vascular damage as well activation of the renin-angiotensin system, conditions which are associated with renal impairment (32,33). In addition, renal function loss can lead to physical decline, frailty and other indirect causes of mortality (34).

The strengths of our study include the long duration of follow-up, the rigorous design of the study, the ability to adjust for a number of important risk factors and the use of linear mixed models to calculate eGFR slope. Conversely, we must also recognize several limitations. These include the use of estimated rather than measured GFR, the lack of ACR values for all patients in the study, the relatively small size of the study cohort. In addition, no information on cardiovascular and

1 cancer mortality was available, thus impeding us to look for the association between GFR decline
2 and these specific causes of death previously reported in type 2 diabetes (29). Finally, the lack of
3 data on new antidiabetic drug classes (SGLT2 inhibitors and GLP-1 analogs) which are known to
4 impact on the risk of death is also a limitation of our study.

5 In summary, we show that in patients with type 2 diabetes, a rapid decline in eGFR is strongly
6 associated with future risk of all-cause mortality. Measuring eGFR slope in the clinical set can
7 therefore be useful to identify those patients who are at high risk of all-cause death and who require
8 close follow-up for the early initiation of preventive and therapeutic strategies. In addition, eGFR
9 decline mediates approximately a quarter of the association between KTR and mortality. This
10 finding points to a novel pathogenic pathway linking tryptophan metabolism, kidney dysfunction
11 and risk of death, thus calling for further studies to identify new treatments tailored to patients
12 whose clinical trajectory is likely to follow this path.

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14 Mortality Study for the dedication and contributions.

15 **Data availability:** Some or all datasets generated during and/or analyzed during the current study
16 are not publicly available but are available from the corresponding author on reasonable request.

References

1. Magliano DJ, Boyko EJ, IDF Diabetes Atlas 10th edition scientific committee. *IDF DIABETES ATLAS*. 10th ed. International Diabetes Federation, Brussel, Belgium: 2021.
2. Rao Kondapally Seshasai S, Kaptoge S, Thompson A, *et al*. Emerging Risk Factors Collaboration. Diabetes mellitus, fasting glucose, and risk of cause-specific death. *N Engl J Med*. 2011;364(9):829-841.
3. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med*. 2008;359(15):1577-1589.
4. UK Prospective Diabetes Study Group. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK Prospective Diabetes Study Group. *BMJ*. 1998;317(7160):703-713.
5. Colhoun HM, Betteridge DJ, Durrington PN, *et al*. Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. *Lancet*. 2004;364(9435):685-696.
6. Rawshani A, Rawshani A, Franzén S, *et al*. Mortality and Cardiovascular Disease in Type 1 and Type 2 Diabetes. *N Engl J Med*. 2017;376(15):1407-1418.
7. Matsushita K, Selvin E, Bash LD, Franceschini N, Astor BC, Coresh J. Change in estimated GFR associates with coronary heart disease and mortality. *J Am Soc Nephrol*. 2009;20(12):2617-2624.
8. Turin TC, Coresh J, Tonelli M, *et al*. Change in the estimated glomerular filtration rate over time and risk of all-cause mortality *Kidney Int*. 2013;83(4):684-691.
9. Al-Aly Z, Zeringue A, Fu J, Rauchman MI, *et al*. Rate of kidney function decline associates with mortality. *J Am Soc Nephrol*. 2010;21(11):1961-1969.

10. Coresh J, Turin TC, Matsushita K, *et al.* Decline in estimated glomerular filtration rate and subsequent risk of end-stage renal disease and mortality. *JAMA*. 2014;311(24):2518-2531.
11. Guo Y, Cui L, Ye P, Li J, Wu S, Luo Y. Change of Kidney Function Is Associated With All-Cause Mortality and Cardiovascular Diseases: Results From the Kailuan Study. *J Am Heart Assoc*. 2018;7(21):e010596.
12. Jiang G, Luk AOY, Tam CHT, *et al.* Progression of diabetic kidney disease and trajectory of kidney function decline in Chinese patients with Type 2 diabetes. *Kidney Int*. 2019;95(1):178-187.
13. Oshima M, Jun M, Ohkuma T, *et al.* The relationship between eGFR slope and subsequent risk of vascular outcomes and all-cause mortality in type 2 diabetes: the ADVANCE-ON study. *Diabetologia*. 2019;62(11):1988-1997.
14. Trischitta V, Mastroianno M, Scarale MG, *et al.* Circulating metabolites improve the prediction of renal impairment in patients with type 2 diabetes. *BMJ Open Diabetes Res Care*. 2023;11(5):e003422.
15. Liu JJ, Ching J, Wee HN, *et al.* Plasma Tryptophan-Kynurenine Pathway Metabolites and Risk for Progression to End-Stage Kidney Disease in Patients With Type 2 Diabetes. *Diabetes Care*. 2023;46(12):2223-2231.
16. Scarale MG, Mastroianno M, Prehn C, *et al.* Circulating Metabolites Associate With and Improve the Prediction of All-Cause Mortality in Type 2 Diabetes. *Diabetes*. 2022;71(6):1363-1370.
17. von Elm E, Altman DG, Egger M, *et al.* The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol*. 2008;61(4):344-349.

- 1 18. Levey AS, Stevens LA, Schmid CH, *et al.* A new equation to estimate glomerular filtration rate.
2 *Ann Intern Med.* 2009;150(9):604-612.
- 3 19. Haid M, Muschet C, Wahl S, *et al.* Long-Term Stability of Human Plasma Metabolites during
4 Storage at -80 °C. *J Proteome Res* 2018;17:203-211.
- 5 20. Stekhoven DJ, Bühlmann P. MissForest--non-parametric missing value imputation for mixed-
6 type data. *Bioinformatics.* 2012;28(1):112-118.
- 7 21. Imai K, Keele L, Tingley D. A general approach to causal mediation analysis. *Psychol Methods.*
8 2010;15(4):309-334.
- 9 22. Lamacchia O, Menzaghi C, Copetti M *et al.* Data from GFR decline predicts total mortality
10 and mediates the effect of tryptophan metabolism on death risk in type 2 diabetes. Figshare.
11 Deposited 26 July 2024 <https://doi.org/10.6084/m9.figshare.26380165.v1>
- 12 23. Chen DQ, Cao G, Chen H, *et al.* Identification of serum metabolites associating with chronic
13 kidney disease progression and anti-fibrotic effect of 5-methoxytryptophan. *Nat Commun.*
14 2019;10(1):1476.
- 15 24. Debnath S, Velagapudi C, Redus L, *et al.* Tryptophan Metabolism in Patients With Chronic
16 Kidney Disease Secondary to Type 2 Diabetes: Relationship to Inflammatory Markers. *Int J*
17 *Tryptophan Res.* 2017 Mar 10;10:1178646917694600.
- 18 25. Goek ON, Prehn C, Sekula P, *et al.* Metabolites associate with kidney function decline and
19 incident chronic kidney disease in the general population. *Nephrol Dial Transplant*
20 2013;28:2131-2138.
- 21 26. Yoshimura H, Sakai T, Kuwahara Y, *et al.* Effects of kynurenine metabolites on mesangial
22 cell proliferation and gene expression. *Exp Mol Pathol* 2009;87:70-75.

27. Pan B, Zhang H, Hong Y, Ma M, Wan X, Cao C. Indoleamine-2,3-dioxygenase activates Wnt/b-catenin inducing kidney fibrosis after acute kidney injury. *Gerontology* 2021;67:611–619.
28. Reyes-Ocampo J, Ramírez-Ortega D, Cervantes GI, *et al.* Mitochondrial dysfunction related to cell damage induced by 3-hydroxykynurenine and 3-hydroxyanthranilic acid: Non-dependent-effect of early reactive oxygen species production. *Neurotoxicology* 2015;50:81–91.
29. Ragot S, Saulnier PJ, Velho G, *et al.* Dynamic Changes in Renal Function Are Associated With Major Cardiovascular Events in Patients With Type 2 Diabetes. *Diabetes Care*. 2016;39(7):1259-1266.
30. Rodriguez-Valadez JM, Tahsin M, Fleischmann KE, *et al.* Cardiovascular and Renal Benefits of Novel Diabetes Drugs by Baseline Cardiovascular Risk: A Systematic Review, Meta-analysis, and Meta-regression. *Diabetes Care*. 2023; 46(6):1300-1310.
31. Bueno Junior CR, Bano A, Tang Y, *et al.* Rapid kidney function decline and increased risk of heart failure in patients with type 2 diabetes: findings from the ACCORD cohort: Rapid kidney function decline and heart failure in T2D. *Cardiovasc Diabetol*. 2023;22(1):131.
32. Schiffrin EL, Lipman ML, Mann JF. Chronic kidney disease: effects on the cardiovascular system. *Circulation*. 2007;116(1):85-97.
33. Navarro-González JF, Mora-Fernández C, Muros de Fuentes M, García-Pérez J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. *Nat Rev Nephrol*. 2011;7(6):327-340.
34. Naimark DM, Grams ME, Matsushita K, *et al.* Past Decline Versus Current eGFR and Subsequent Mortality Risk. *J Am Soc Nephrol*. 2016;27(8):2456-2466.

Figure 1: Mortality by tertiles of eGFR decline. Left (Panel A) incidence rate of mortality per tertile. The incidence rate is given x 100 person-years after adjustment for age and sex. Right (Panel B) Kaplan-Meier survival curve: tertile 3 in black, tertile 2 in dark grey and tertile 1 in light grey.

Figure 2: Association between eGFR decline and mortality in subgroups of demographical and clinical features. Hazard ratios (HR) and 95% CI per each ml/min increase in the annual rate of eGFR loss were estimated by Cox regression models. The p value for heterogeneity is shown for each subgroup.

Table 1. Clinical features of the entire aGMS cohort (N =2,140), the 962 study individuals and the 575 subjects for metabolomics study

	N = 2,140	N = 962	N = 575
Women n (%)	1145 (53.5)	455 (47.3)	275 (47.8)
Age at recruitment (years)	62.0 ± 9.42	60.8 ± 9.7	60.9 ± 9.8
Smokers n (%)	310 (14.5)	148 (15.4)	79 (13.7)
BMI (kg/m ²)	31.0 ± 5.8	31.2 ± 5.8	31.2 ± 6.0
Diabetes duration (years)	11.2 ± 9.0	10.8 ± 9.0	11.6 ± 9.3
HbA1c (%)	8.5 ± 1.9	8.5 ± 1.9	8.4 ± 1.9
HbA1c mmol/mol	69 ± 20.8	69 ± 20.8	68 ± 20.8
Systolic blood pressure (mmHg)	134.7 ± 17.5	134.2 ± 17.2	134.4 ± 17.0
Diastolic blood pressure (mmHg)	78.6 (9.21)	78.8 (9.15)	78.8 (8.90)
Total cholesterol (mg/dl)	188.9 ± 49.8	190.6 ± 55.7	189.5 ± 56.2
HDL cholesterol (mg/dl)	44.6 ± 12.7	44.5 ± 13.0	44.2 ± 12.7
Triglycerides (mg/dl)	128.0 [91.0- 181.0]	128.0 [89.0- 180.8]	128.0 [90.0- 180.0]
Insulin (w/wo other glucose-lowering agents) n (%)	894 (41.8)	400 (41.6)	268 (46.6)
Lipid-lowering TX n (%)	1016 (47.5)	419 (43.6)	292 (50.8)
Blood pressure-lowering TX (%)	1418 (66.3)	600 (62.4)	376 (65.4)
eGFR (ml/min/1.73 m ²)	80.9 ± 18.7	79.2 ± 21.5	77.4 ± 26.1
ACR (mg/mmol)*	2.07 [0.82-6.27]	1.38 [0.68- 3.70]	1.56 [0.70-4.96]
Annual rate of eGFR decline**	1.3 (-3.7-7.8)	1.3 (-3.7-7.8)	1.3 (-2.2-0.6)
All-cause death (n) (%)	1067 (49.9)	452 (47.0)	227 (39.5)
Follow-up (years); (py)	13.7 ± 5.8 (29,285)	14.8 ± 5.5 (14,197)	13.3 ± 5.2 (7,670)
IR (n events per 100 py) (95% CI) †	3.32 (3.1-3.55)	2.75 (2.5-3.1)	2.46 (2.11-2.88)

1 Continuous variables were reported as mean ± SD or median whereas categorical variables as total frequencies and percentages. Skewed
2 continuous variables are presented as median [range], BMI (body mass index), HbA1c (glycated hemoglobin), eGFR (estimated glomerular
3 filtration rate, calculated using CKD-EPI equation), ACR (albumin-creatinine ratio).

4 *Information on albuminuria was available for only 1,529 patients out of the total 2,140-patient group, for 637 patients out of the total 962-patient
5 group and for all subjects in the 575-patient group.

6 **In the whole cohort, comprising 2140 patients, annual rate of eGFR decline was available only in the 962 study individuals used in this analysis.

7 †IR: Incidence rate of mortality is given x 100 person-years, after adjusting for age and gender.

8 Data above the dotted line have been collected at baseline, while those below the dotted line refer to the follow-up and incidence death, the study
9 outcome.

Table 2. Uni- and Multivariable Cox regression model for the risk of mortality – Role of baseline variables

	Univariate Analysis		Multivariate Analysis	
	HR (95%CI)	p-value	HR (95%CI)	p-value
Annual rate of decline in eGFR (one ml/min per 1.73 m ²)	1.43 (1.34-1.53)	<0.0001	1.31 (1.21-1.41)	<0.001
Gender (male vs female)	1.14 (0.95-1.38)	0.1542	1.68 (1.38 -2.04)	<0.0001
Age at recruitment (one year)	1.10 (1.09-1.11)	<0.0001	1.08 (1.07-1.09)	<0.0001
BMI (one Kg/m ²)	1.01 (1.00-1.03)	0.1506	1.02 (1.00-1.04)	0.02
Diabetes duration (one year)	1.05 (1.04-1.06)	<0.0001	1.01 (1.00-1.02)	0.10
HbA1c (one unit %)	1.13 (1.08-1.18)	<0.0001	1.13 (1.07-1.19)	<0.0001
eGFR (one ml/min per 1.73 m ²)	0.97 (0.97-0.97)	<0.0001	0.99 (0.98-0.99)	<0.0001
Insulin (w/wo other glucose-lowering agents) (yes vs no)	2.76 (2.29-3.34)	<0.0001	1.79 (1.43-2.23)	<0.001
Lipid-lowering TX (yes vs no)	1.50 (1.24-1.82)	<0.0001	1.01 (0.82-1.24)	0.96
Blood pressure-lowering TX (yes vs no)	2.00 (1.63-2.45)	<0.0001	1.17 (0.93-1.49)	0.19

BMI (body mass index), HbA1c (glycated hemoglobin), eGFR (estimated glomerular filtration rate, calculated using CKD-EPI equation). HRs (per ml/min increase in the annual rate of eGFR loss) were estimated in Cox regression model, adjusted for gender, age at recruitment, HbA1c, BMI, eGFR, diabetes duration, insulin (w/wo other glucose-lowering agents), lipid-lowering TX, blood pressure-lowering TX and recruitment period.

Table 3. Risk of all-cause mortality by tertiles of eGFR decline

		HR (95%CI)	p-value
Model 1	T1	1	
	T2	2.19 (1.69-2.82)	<0.001
	T3	3.14 (2.45-3.99)	<0.001
Model 2	T1	1	
	T2	1.45 (1.11-1.87)	<0.001
	T3	1.81 (1.40-2.34)	<0.001

eGFR decline was -3.74 -- 0.79; 0.79 -- 1.85; 1.85 -- 7.77 in tertile 1 (T1), 2 (T2) and 3 (T3), respectively (negative values indicate improved eGFR at follow-up). The rate of death in tertile 1 is the reference (HR made equal to 1).

Model 1: adjusted only for the recruitment period.

Model 2: adjusted for gender, age at recruitment, HbA1c, BMI, eGFR, diabetes duration, insulin (w/wo other glucose-lowering agents), lipid-lowering TX, blood pressure-lowering TX and the recruitment period.

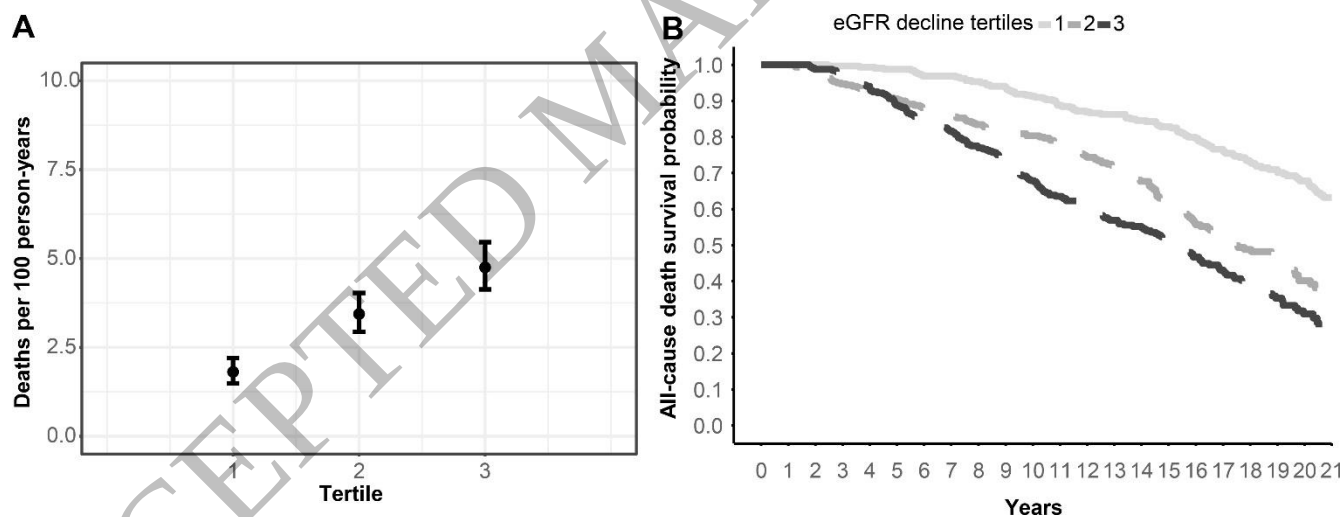


Figure 1
176x66 mm (DPI)

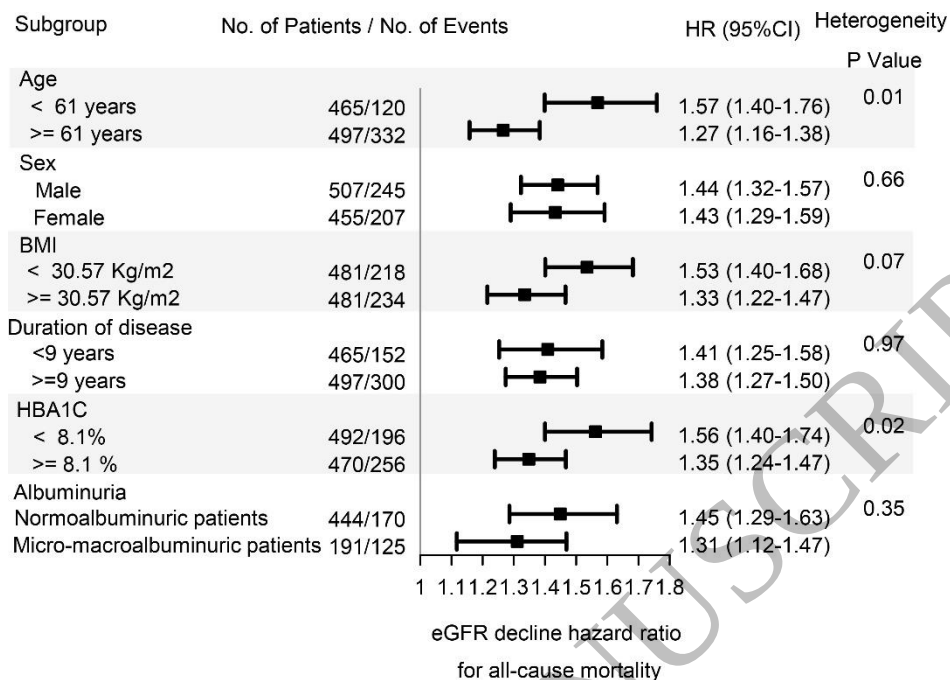


Figure 2
124x88 mm (DPI)