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Filtration Markers, Cardiovascular Disease, Mortality, and Kidney Outcomes in Stable Kidney Transplant Recipients: The FAVORIT Trial

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Abstract

Cystatin C and beta-2-microglobulin (B2M) are filtration markers associated with adverse outcomes in non-transplant populations, sometimes with stronger associations than for creatinine. We evaluated associations of estimated glomerular filtration rate from cystatin C ($eGFR_{cys}$), B2M ($eGFR_{B2M}$), and creatinine ($eGFR_{cr}$) with cardiovascular outcomes, mortality, and kidney failure in stable kidney transplant recipients using a case-cohort study nested within the Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) Trial. A random subcohort was selected (N=508; mean age 51.6 years, median transplant vintage 4 years, 38% women, 23.6%

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Supporting Information

Additional Supporting Information may be found in the online version of this article

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non-white race) with enrichment for cardiovascular events (N=306; 54 within the subcohort), mortality (N=208; 68 within the subcohort), and kidney failure (N=208; 52 within the subcohort). Mean $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{B2M}$ were 46.0, 43.8, and 48.8 mL/min/1.73m^2, respectively. After multivariable adjustment, hazard ratios for $eGFR_{cys}$ and $eGFR_{B2M}$ <30 vs. 60+ were 2.02 (95% CI 1.09–3.76; p=0.03) and 2.56 (1.35–4.88; p=0.004) for cardiovascular events; 3.92 (2.11–7.31) and 4.09 (2.21–7.54; both p<0.001) for mortality; and 9.49 (4.28–21.00) and 15.53 (6.99–34.51; both p<0.001) for kidney failure. Associations persisted with additional adjustment for baseline $eGFR_{cr}$. We conclude that cystatin C and B2M are strongly associated with cardiovascular events, mortality, and kidney failure in stable kidney transplant recipients.

Introduction

Kidney transplant recipients have increased risk of cardiovascular disease, kidney failure and all-cause mortality. Lower levels of kidney function, ascertained with serum creatinine or glomerular filtration rate (GFR) estimated from serum creatinine ($eGFR_{cr}$) is independently associated with cardiovascular and kidney disease outcomes in a wide range of non-transplant populations,(1–4) and is associated with increased risk of cardiovascular events(5–9), all-cause mortality,(10) and graft loss(11–13) in kidney transplant recipients.

Cystatin C and beta-2 microglobulin (B2M) are low molecular weight serum proteins that are being investigated as novel filtration markers. In non-transplant populations, these filtration markers and their related GFR estimates ($eGFR_{cys}$, $eGFR_{B2M}$) are independent predictors of mortality, cardiovascular events, and ESRD, and in some cases show stronger risk associations than those observed for serum creatinine and $eGFR_{cr}$.(14–21) It is hypothesized that such differences in risk associations among filtration markers reflect differences in factors other than GFR that influence their serum levels. The non-GFR determinants of filtration markers may differ in kidney transplant recipients compared to the non-transplant population, leading to differences in risk associations between these populations. Few studies have evaluated risk associated with low molecular weight serum protein levels in kidney transplant recipients. Prior work suggests that higher B2M is associated with greater eGFR decline 1–2 years post-transplant(22) and that both higher cystatin C and B2M are associated with increased risk of mortality(23) and graft loss(23–25) in transplant recipients. It is not known, however, whether the potential advantage of the low molecular weight serum protein filtration markers over creatinine for estimating cardiovascular and kidney disease risk associations that have been observed in the non-transplant population are present among transplant patients. If the cystatin C and B2M are shown to be strong markers of major clinical outcomes in transplant recipients, they may prove useful in risk prediction and risk stratification in this population.

We have previously demonstrated an independent association of baseline $eGFR_{cr}$ with cardiovascular disease and all-cause mortality in a post-hoc analysis of the Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) Trial.(8) The aim of the current study is to evaluate risk associations of $eGFR_{cys}$ and $eGFR_{B2M}$ with cardiovascular outcomes, all-cause mortality, and kidney failure in stable kidney transplant recipients in a

case-cohort study drawn from the FAVORIT Trial, and whether these associations are independent of $eGFR_{cr}$.

Materials and Methods

Study population

Our study sample was drawn from the FAVORIT Trial (clinicaltrials.gov: NCT00064753), a multi-center double-blind randomized clinical trial conducted to determine whether lowering homocysteine levels with vitamin therapy reduced the rate of cardiovascular outcomes.(26, 27) The FAVORIT Study was approved by the Institutional Review Boards at participating institutions. All participants provided written informed consent. In brief, 4,110 kidney transplant recipients aged 35–75 years who were at least 6 months post-kidney transplant were enrolled between August 2002 and January 2007 at 30 transplant centers in the United States, Canada, and Brazil. Trial eligibility required an elevated serum homocysteine level (≥11μmol/L [women]; ≥12μmol/L [men]) and stable kidney function (estimated creatinine clearance ≥30mL/min [men] or ≥25mL/min [women]). Participants were followed-up every six months through January 31, 2010 to obtain outcomes through June 24, 2009.

For the present analysis we utilized an established case-cohort study nested within the FAVORIT Trial. The case-cohort study is an efficient design to evaluate novel factors associated with multiple outcomes with a study sample. It includes (1) a random sample of participants drawn from the overall original study (the “sub-cohort”) that is additionally enriched to include (2) all outcomes of interest that occur within the overall original study population. For the FAVORIT case-cohort study, a sub-cohort was selected to include a 15% random sample of participants with non-missing baseline measurements of serum creatinine, $eGFR_{cr}$, cholesterol, and triglycerides, no missing follow-up, and stored serum and urine samples available (a key component of the case-cohort study for additional novel biomarker measurements); 3530 of the 4110 participants in the overall FAVORIT trial cohort met these criteria, 530 of whom were selected for the sub-cohort.(28–30) For the present analyses, we additionally excluded participants in the sub-cohort with missing serum measurements of cystatin C and B2M or key model covariates at baseline (N=22). Weighted Cox proportional hazards regression analyses for adjudicated cardiovascular events included 306 participants with events (54 events that overlap with the sub-cohort), resulting in an analytic study sample of 760 participants. Analyses for all-cause mortality included 382 participants with events (68 events that overlap with the sub-cohort), resulting in an analytic sample of 822 participants. Analyses for dialysis-dependent kidney failure included 280 participants with events (52 events that overlap with the sub-cohort), resulting in an analytic sample of 736 participants.

Exposure Assessment

Serum creatinine was previously measured in 2011 from frozen samples collected during the baseline study visit following a single thaw. Serum creatinine assays were performed at the United States Department of Agriculture Jean Mayer Human Nutrition Research Center on Aging at Tufts University using an alkaline picrate kinetic method on an Olympus AU 400e (Olympus America Inc, Center Valley, PA) instrument (coefficient of variation [CV] intra

assay 2.0%; -iInter assay 4.0%), calibrated to an isotope dilution mass spectrometry traceable standard.(8) Serum cystatin C and B2M were measured at the Brigham and Women's Hospital in 2012 in previously frozen baseline EDTA-treated plasma samples using a Roche Cobas C6000 analyzer (Roche Diagnostics, Indianapolis, IN). Cystatin C assays were calibrated to an IFCC standard. Total imprecision of the B2M assay was 1.7% at 2.42 mg/L and 1.9% at 5.60 mg/L. Total imprecision of the cystatin C assay was 3.7% at 0.78 mg/L and 2.0% at 3.43 mg/L. Filtration markers were transformed to eGFR using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine (2009), cystatin C (2012), and B2M (2015) estimation equations(31–33) to allow comparisons across markers on the same scale (mL/min/1.73m^2).

Outcome Assessment

Outcomes included adjudicated cardiovascular events, all-cause mortality, and dialysis-dependent kidney failure. Adjudicated cardiovascular events included cases of cardiovascular death, myocardial infarction, resuscitated sudden death, and stroke that were centrally adjudicated and reviewed by the FAVORIT Clinical Endpoints Committee. Dialysis-dependent kidney failure was defined as the need for chronic dialysis as ascertained by local study staff.

Covariate Assessment

Demographic, clinical, and transplant characteristics were assessed at the time of study enrollment. Smoking status was defined based on self-report and categorized as current, former or never smoking status. Transplant characteristics included donor type (living vs. deceased donor) and vintage (years since transplant). Baseline systolic and diastolic blood pressure was defined as the average of two measurements. BMI was calculated as weight/height^2 (kg/m^2). Diabetes was defined as the use of insulin or oral hypoglycemic medications or based on self-report. Self-reported prevalent cardiovascular disease at baseline included prior myocardial infarction, coronary artery revascularization, stroke, carotid arterial revascularization, abdominal or thoracic aortic aneurysm repair, and/or lower extremity arterial revascularization or non-traumatic amputation above the ankle. Additional laboratory measurements included serum cholesterol measurements.

Statistical Analyses

Baseline demographic and clinical characteristics are presented for the sub-cohort of 508 participants, overall and by $eGFR_{cys}$ and $eGFR_{B2M}$ categories. We calculated Pearson correlation coefficients between $eGFR_{cys}$, $eGFR_{B2M}$, and $eGFR_{cr}$ within the sub-cohort. Associations of $eGFR_{cys}$, $eGFR_{B2M}$, and $eGFR_{cr}$ with adjudicated cardiovascular events, all-cause mortality, and dialysis-dependent kidney failure were first evaluated using Kaplan-Meier survival curves with log-rank tests in the sub-cohort (N=508). We then used weighted Cox proportional hazards regression in the full analytic samples for each outcome to evaluate the association of $eGFR_{cys}$, $eGFR_{B2M}$, and $eGFR_{cr}$ with events to account for the weighted study design, similar to prior work in this case-cohort.(28–30, 34) Model adjustment included (1) adjustment for demographic and transplant characteristics (age, sex, race, country, randomized treatment assignment, donor type, graft vintage); (2) additional multivariable adjustment for baseline cardiovascular disease, diabetes, smoking status, LDL

cholesterol, HDL cholesterol, triglycerides, systolic blood pressure, diastolic blood pressure, body mass index, and natural log-transformed urinary albumin to creatinine ratio; and (3) additional adjustment for $eGFR_{cr}$ in models with $eGFR_{cys}$ or $eGFR_{B2M}$ as the primary predictor of interest. Exposures were modeled categorically and continuously in analyses. Statistical analyses were performed in Stata, Version 12.1 (StataCorp LP) and SAS Version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Baseline Characteristics of the Sub-Cohort

In the sub-cohort (n=508), mean age was 51.6±9.2 years, 38.0% were female, 23.6% were of non-white race, and 68.5%, 13.5%, and 17.9% were recruited from centers in the United States, Canada, and Brazil, respectively (Table 1); characteristics of the sub-cohort are similar to those observed in the larger FAVORIT study cohort.(8) Participant characteristics across $eGFR_{cys}$, $eGFR_{B2M}$, and $eGFR_{cr}$ groups are presented in Tables 1, S1, and S2, respectively. A strong correlation was observed between $eGFR_{cys}$ and $eGFR_{B2M}$ (Pearson correlation coefficient=0.87, $p<0.001$); $eGFR_{cys}$ and $eGFR_{B2M}$ were more moderately correlated with $eGFR_{cr}$ (Pearson correlation coefficients of 0.61 and 0.55, respectively, both $p<0.001$). Table S3 compares demographic characteristics and outcomes in the random sub-cohort to those who were not included in the subcohort. Serum creatinine was significantly higher and there was a trend toward a higher proportion of participants who developed kidney failure in participants selected in the sub-cohort.

Adjudicated Cardiovascular Events

Over median follow-up of 3.6 years in the sub-cohort, 54 (10.6%) adjudicated cardiovascular events occurred. Kaplan-Meier survival analyses plots of the relationship between $eGFR_{cys}$, $eGFR_{B2M}$, or $eGFR_{cr}$ and cardiovascular outcomes in the sub-cohort are presented in Figure 1. In the case-cohort analysis in the full cardiovascular events analytic sample (N=760) with weighted Cox proportional hazards regression, lower $eGFR_{cys}$ and lower $eGFR_{B2M}$ were each significantly associated with an increased risk of cardiovascular events after adjustment for demographic and transplant characteristics (Table 2; both p-trend<0.001 when modeled categorically and $p<0.001$ when modeled continuously). Results were similar with additional multivariable adjustment (HR for eGFR<30 vs. eGFR 60+ for $eGFR_{cys}$ and $eGFR_{B2M}$ of 2.02 [95% CI 1.09–3.76; p=0.03] and 2.56 [95% CI 1.35–4.88; p=0.004]), and associations of $eGFR_{cys}$ and $eGFR_{B2M}$ with cardiovascular events persisted in models that further adjusted for $eGFR_{cr}$. Lower $eGFR_{cr}$ categories were associated with a trend for increased risk of cardiovascular events with demographic and transplant characteristic adjustment (p-trend=0.02), although this was not significant after additional multivariable adjustment (p-trend=0.32); no significant associations were observed with continuous $eGFR_{cr}$ (Table 2).

All-Cause Mortality

Over median follow-up of 3.8 years, 68 (13.3%) participants within the sub-cohort died. In Kaplan-Meier survival analyses, significant differences were observed across $eGFR_{cys}$ and $eGFR_{B2M}$ but not $eGFR_{cr}$ categories within the sub-cohort (Figure 2). In the case-cohort

analysis in the full all-cause mortality analytic sample (N=822) with weighted Cox proportional hazards regression, lower $eGFR_{cys}$ and lower $eGFR_{B2M}$ were each significantly associated with an increased all-cause mortality risk after accounting for demographic and transplant characteristics (Table 3; both p-trend<0.001 when modeled categorically and p<0.001 when modeled continuously). Results were similar with additional multivariable adjustment and persisted in models that further adjusted for $eGFR_{cr}$ (Table 3; HR for eGFR<30 vs. eGFR 60+ 3.92 [95% CI 2.11–7.31] and 4.09 [95% CI 2.21–7.54]; both p<0.001). In contrast, no significant trend or associations were observed with all-cause mortality for $eGFR_{cr}$ when modeled categorically or continuously.

Dialysis-Dependent Kidney Failure

Over a median follow-up of 3.6 years, 52 (10.2%) of participants in the sub-cohort experienced dialysis-dependent kidney failure. In Kaplan-Meier survival analyses significant differences were observed across $eGFR_{cys}$, $eGFR_{B2M}$, and $eGFR_{cr}$ groups curves within the sub-cohort (all log-rank p<0.001; Figure 3). In case-cohort analyses in the full kidney failure analytic sample (N=736) with weighted Cox proportional hazards regression, lower eGFR based on all three filtration markers was associated with increased risk of kidney failure when modeled categorically (all p-trend<0.001) or continuously (all p<0.001, Table 4) after accounting for demographic and transplant characteristics. Results were similar with additional multivariable adjustment (HR for eGFR<30 vs. eGFR 60+ of 9.49 [95% CI 4.28–21.00] for $eGFR_{cys}$ and 15.53 [95% CI 6.99–34.51] for $eGFR_{B2M}$; both p<0.001), and persisted for both $eGFR_{cys}$ and $eGFR_{B2M}$ in models that further adjusted for $eGFR_{cr}$ (Table 4).

Discussion

Lower eGFR based on the low molecular weight serum proteins cystatin C and B2M is strongly associated with cardiovascular events, mortality, and dialysis-dependent kidney failure in stable kidney transplant recipients from the FAVORIT study. These associations persist following adjustment for several established risk factors and were independent of $eGFR_{cr}$. As strong independent predictors of cardiovascular, mortality, and kidney events these filtration markers are of interest as potential factors that could contribute to risk prediction in this population.

Overall our findings are consistent with results for cystatin C and B2M observed in non-transplant populations(14–21) and extend the current literature of the strong risk factor associations of these filtration markers with important outcomes into the kidney transplant population. Few studies have evaluated risk associated with cystatin C and B2M in kidney transplant recipients.(22–25) In small studies (sample size from 79 to 129) higher cystatin C levels were associated with graft loss(24, 25) and higher serum B2M levels were also associated with $eGFR_{cr}$ decline at 1 and 2 years follow-up.(22) In the largest study to date, Astor and colleagues reported that higher serum B2M levels at discharge were associated with both mortality and graft loss and with associations of greater magnitude when compared to serum creatinine in a cohort of 2190 primary kidney transplant recipients from a single center in the United States.(23) Our findings extend the current literature in kidney

transplant recipients by confirming that cystatin C and B2M are strongly associated with mortality and dialysis-dependent kidney failure in a diverse cohort of stable kidney transplant recipients from the United States, Canada, and Brazil, in addition to our novel findings related to increased risk of cardiovascular events.

It is difficult to compare the magnitude of the risk associations that we observed for $eGFR_{cys}$ and $eGFR_{B2M}$ with those for $eGFR_{cr}$. The HRs for $eGFR_{cys}$ and $eGFR_{B2M}$ with cardiovascular events, mortality and dialysis-dependent kidney failure are larger than those observed for $eGFR_{cr}$ in the present case-cohort analysis as well as those observed in a previous study in the full FAVORIT cohort.(8) In the previous study, the HRs for $eGFR_{cr}$ with cardiovascular disease and all-cause mortality were modestly stronger than in this study and statistically significant, although current estimates fall within prior 95% CIs with substantial overlap. These differences may be related to the sampling design in the case-cohort study, which may reduce the relative efficiency of the $eGFR_{cr}$ analyses compared to the full cohort. A different reference group was also selected in the previous study ($eGFR_{cr}$ 60–75mL/min/1.73m^2) whereas we combined the two highest categories of 60–75 and 75+ for more stable estimates due to our smaller size. Given the null findings in the 75+ compared to the reference group 60–75mL/min/1.73m^2 in the previous study it is less likely that this difference explains the $eGFR_{cr}$ associations observed here. Our interpretation is that the risk associations of $eGFR_{cys}$ and $eGFR_{B2M}$ with these outcomes are likely as strong, and possibly stronger, than for $eGFR_{cr}$.

Risk associations of $eGFR_{cys}$, $eGFR_{B2M}$, and $eGFR_{cr}$ with important adverse outcomes reflect risk related both to GFR and to non-GFR determinants of the serum levels of the filtration markers. For creatinine, muscle mass is the main non-GFR determinant, and age, sex, and race are incorporated into $eGFR_{cr}$ as surrogates in populations where major deviations in muscle mass are not expected. Factors that reduce muscle mass, such as chronic illness and glucocorticosteroid use, may lead to overestimation of GFR and consequent attenuation of risk associations with $eGFR_{cr}$.

The non-GFR determinants of cystatin C and B2M are not as well understood. Cystatin C is a 120 amino acid serine protease inhibitor that serves many biologic functions. In non-transplant populations, factors associated with non-GFR determinants of cystatin C include larger body size, inflammation, male sex, and smoking.(35–38) Results from *in vitro* studies indicate that cystatin C is generated by immune cells and adipose tissue, consistent with variation in generation of cystatin C independent of GFR.(39, 40) As in the non-transplant population, obesity and inflammation may affect serum levels of cystatin C in kidney transplant recipients and contribute to increased risk of adverse outcomes independent of decreased GFR. In kidney transplant recipients, recent work suggests that multiple cardiovascular risk factors are associated with $eGFR_{cys}$ independent of measured GFR, including higher age, diabetes, smoking, CVD, dialysis, higher BMI, higher serum triglycerides, higher urine protein, and higher hemoglobin A1c; representing a larger number of non-GFR determinants associated with cystatin C when compared to creatinine.(41) This suggests that the differential risk associations observed in our study may reflect risk related to non-GFR determinants of cystatin C rather than kidney function.

B2M is a 100-amino acid protein that is a component of class I major histocompatibility molecules and is present on the surface of nucleated cells. B2M is higher in some lymphoproliferative and plasma cell disorders and has been used as a biomarker in these conditions.(42, 43) Previous studies have investigated serum B2M in kidney transplant recipients and maintenance hemodialysis patients. In kidney transplant recipients, serum B2M has been studied as a marker of allograft rejection(23) and cytomegalovirus infection(44), but results are confounded by its strong relationship to GFR. In patients treated with maintenance hemodialysis, higher serum B2M has been associated with increased all-cause and infectious mortality risk.(45, 46) While non-GFR determinants of B2M have not been evaluated in kidney transplant recipients, in maintenance hemodialysis patients factors associated with higher levels of pre-dialysis serum B2M included lower age, black race, diabetes, lower BMI, longer dialysis duration, lower residual kidney function, and lower dialysis B2M clearance.(45) In non-transplant and non-dialysis populations, factors most strongly associated with non-GFR determinants of B2M include higher urine protein, smoking, lower serum albumin, diabetes, and higher C-reactive protein, with weaker associations observed for lower age, male sex, non-black race, and higher body mass index. (38) It is possible that some of the mechanisms underlying these associations also contribute to the increased risk observed with B2M levels independent of GFR in kidney transplant recipients.

Our study has several limitations. Our study sample was drawn from a selected sample of stable kidney transplant recipients with decreased kidney function and elevated serum homocysteine and may not be representative of the general kidney transplant population. The case-cohort design utilized a 15% random sample to estimate the exposure distribution in the overall study. Although demographic characteristics in the random sub-cohort are similar to those observed in previous analyses in the overall cohort, we did observe that serum creatinine was higher in participants selected in the sub-cohort compared to those who were not included in the sub-cohort from the overall population (Table S3) and we cannot rule out the impact of sub-cohort selection on the differences in $eGFR_{cr}$ with outcomes observed in the overall cohort, where $eGFR_{cr}$ was statistically associated with cardiovascular disease and all-cause mortality.(8) We also observed a non-significant increased incidence of dialysis dependent kidney failure in participants selected in the sub-cohort compared to those who were not included in the sub-cohort (Table S3), which may impact the generalizability of the findings to the overall kidney transplant population. Cystatin C and B2M were measured once in previously frozen baseline serum samples. Serum levels may vary over time, but we anticipate that variability would be non-differential with respect to outcome assessment and would likely bias our results towards the null. The B2M assay method performed at Brigham and Women's Hospital has not been directly compared to the method used at the University of Minnesota, where the CKD-EPI $eGFR_{B2M}$ equation was developed. We did not assess all potential factors associated with non-GFR determinants of filtration markers, such as CRP, which contribute to the association of filtration markers with outcomes. Finally, $eGFR_{cys}$ and $eGFR_{B2M}$ equations were not developed in the kidney transplant population, which may lead to bias and imprecision in estimates based on different filtration markers; however this effect is likely non-differential with respect to outcome assessment, potentially biasing associations towards the null.

This study has a number of strengths. Our study sample was drawn from a multi-national, well-characterized cohort of stable kidney transplant recipients with long-term follow-up for adjudicated cardiovascular events, all-cause mortality, and dialysis-dependent kidney failure. The case-cohort study design is an efficient methodological approach for studying associations of novel factors with risk for multiple outcomes within a large existing cohort.

In conclusion, we found that the eGFR based on the low molecular weight serum protein filtration markers cystatin C and B2M are independent predictors of cardiovascular events, mortality, and dialysis-dependent kidney failure in stable kidney transplant recipients from the FAVORIT trial. Further work is needed to characterize non-GFR determinants of cystatin C and B2M and to evaluate the impact of LMW serum protein filtration markers on risk prediction beyond creatinine in kidney transplant recipients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

B2M	beta-2-microglobulin
CI	confidence interval
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CVD	cardiovascular disease
$eGFR_{cys}$	estimated glomerular filtration rate from cystatin
$eGFR_{B2M}$	estimated glomerular filtration rate from beta-2-microglobulin
$eGFR_{cr}$	estimated glomerular filtration rate from creatinine
ESRD	end-stage renal disease
HR	hazard ratio
LMW	low molecular weight
***n* or N**	number in group
P	probability
yr	year

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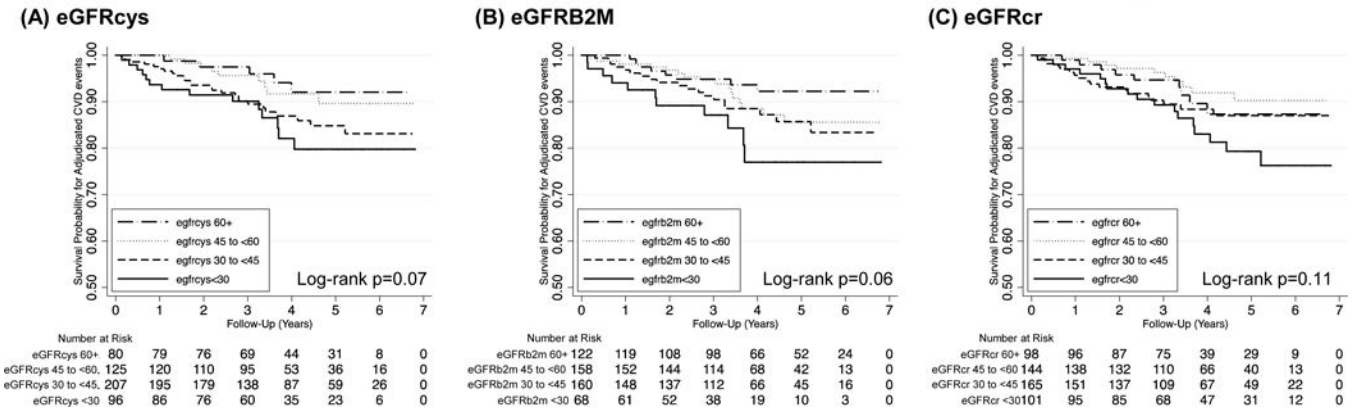

Figure 1.

Kaplan Meier survival curves for adjudicated cardiovascular events in the sub-cohort, by (A) cystatin C-based eGFR, (B) beta-2 microglobulin-based eGFR, and (C) creatinine-based eGFR. The sub-cohort includes 508 participants with 54 adjudicated cardiovascular events. eGFR, estimated glomerular filtration rate.

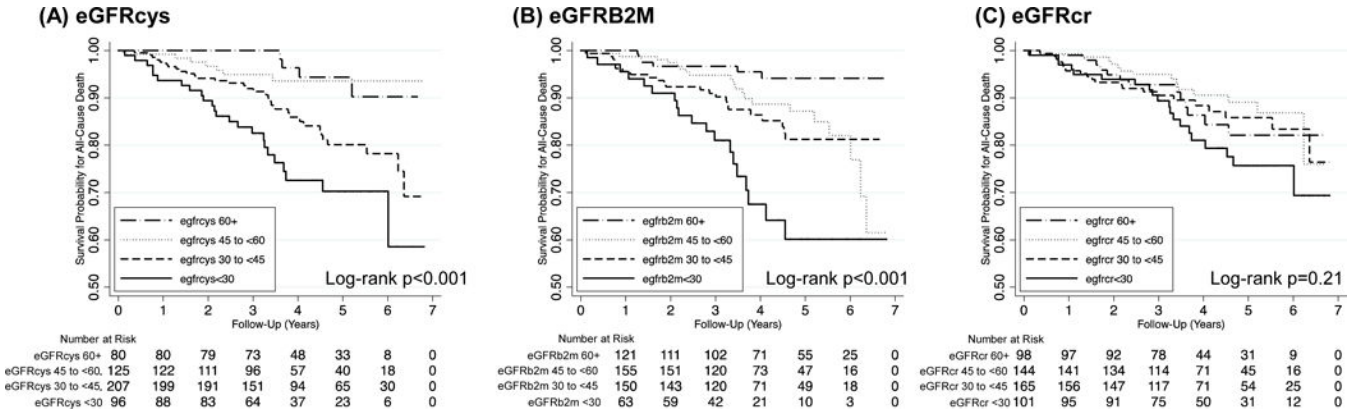

Figure 2.

Kaplan Meier survival curves for all-cause mortality in the sub-cohort, by (A) cystatin C-based eGFR, (B) beta-2 microglobulin-based eGFR, and (C) creatinine-based eGFR. The sub-cohort includes 508 participants with 68 all-cause mortality events. eGFR, estimated glomerular filtration rate.

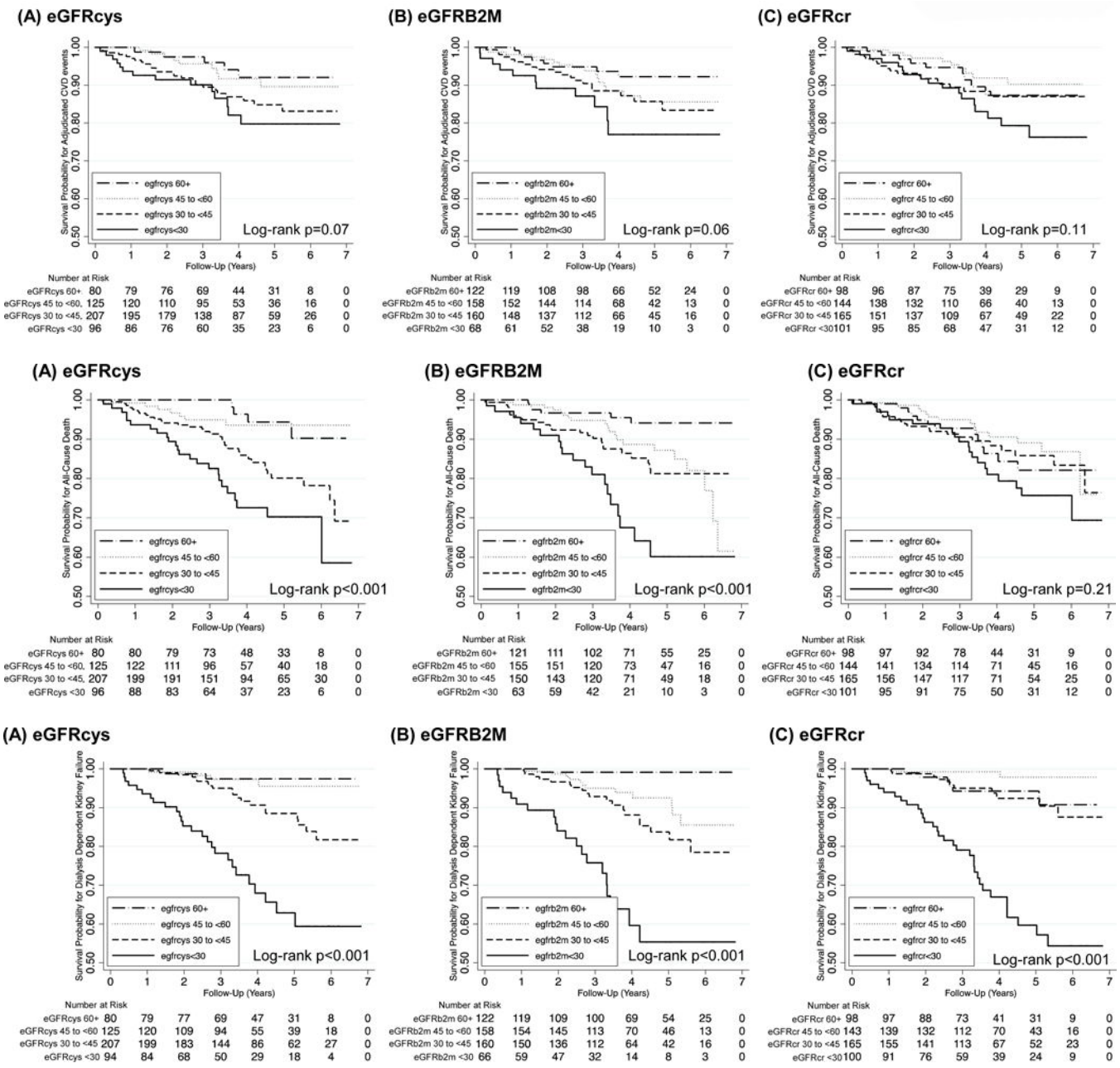

Figure 3.

Kaplan Meier survival curves for kidney failure events in the sub-cohort, by (A) cystatin C-based eGFR, (B) beta-2 microglobulin-based eGFR, and (C) creatinine-based eGFR. The sub-cohort includes 508 participants with 52 kidney failure events. eGFR, estimated glomerular filtration rate.

Table 1

Baseline characteristics of the FAVORIT sub-cohort, overall and by cystatin C-based estimated glomerular filtration rate ($eGFR_{cys}$) group.

Characteristics	Overall	$eGFR_{cys}$ Group (mL/min/1.73m^2)			
		<30	30 to <45	45 to <60	60+
N	508	96	207	125	80
Age	51.6 (9.2)	52.0 (8.7)	52.6 (9.7)	51.0 (8.9)	49.4 (8.6)
Women	193 (38.0%)	40 (41.7%)	83 (40.1%)	42 (33.6%)	28 (35.0%)
Race					
White	388 (76.4%)	70 (72.9%)	157 (75.9%)	97 (77.6%)	64 (80%)
Black	87 (17.1%)	16 (16.7%)	39 (18.8%)	21 (16.8%)	11 (13.75%)
Other	33 (6.5%)	10 (10.4%)	11 (5.3%)	7 (5.6%)	5 (6.25%)
Treatment Group					
High Dose Vitamin	253 (49.8%)	47 (48.96%)	110 (53.1%)	58 (46.4%)	38 (47.5%)
Low Dose Vitamin	255 (50.2%)	49 (51.0%)	97 (46.9%)	67 (53.6%)	42 (52.5%)
Country					
United States	348 (68.5%)	63 (65.6%)	142 (68.6%)	88 (70.4%)	55 (68.75%)
Canada	69 (13.5%)	13 (13.5%)	24 (11.6%)	14 (11.2%)	18 (22.5%)
Brazil	91 (17.9%)	20 (20.8%)	41 (19.8%)	23 (18.4%)	7 (8.75%)
Graft vintage (yr), median (25th, 75th)	4 [2, 7]	5 [2, 9]	4 [2, 7]	3 [1, 5]	4 [2, 7]
Living donor kidney (%)	214 (42.1%)	28 (29.2%)	83 (40.1%)	67 (53.6%)	36 (45.0%)
History of cardiovascular disease (%)	96 (18.9%)	25 (26.0%)	37 (17.9%)	25 (20.0%)	9 (11.25%)
History of diabetes mellitus (%)	184 (36.2%)	38 (39.6%)	69 (33.3%)	53 (42.4%)	24 (30.0%)
Smoking (%)					
Never	254 (50.0%)	48 (50%)	100 (48.3%)	62 (49.6%)	44 (55.0%)
Current	56 (11.0%)	15 (15.6%)	26 (12.6%)	10 (8.0%)	5 (6.25%)
Former	192 (37.8)	32 (33.3%)	79 (38.2%)	51 (40.8%)	30 (37.5%)
Body mass index (kg/m^2)	28.9 (5.9)	29.2 (5.7)	29.6 (6.4)	28.6 (5.5)	27.2 (4.9)
SBP (mm Hg)	136 (20)	138 (24)	136 (20)	137 (19)	129 (17)
DBP (mm Hg)	79 (13)	80 (14)	80 (14)	79 (10)	78 (11)

Characteristics	Overall	$eGFR_{cys}$ Group (mL/min/1.73m^2)			
		<30	30 to <45	45 to <60	60+
Total Cholesterol (mg/dL)	188 (43)	190 (51)	188 (44)	186 (38)	187 (35)
LDL cholesterol (mg/dL)	103 (33)	102 (37)	103 (33)	104 (32)	104 (31)
HDL cholesterol (mg/dL)	47 (14)	42 (16)	47 (13)	47 (14)	50 (15)
Triglycerides (mg/dL)	167 [114, 243]	189 [142, 274]	167 [117, 240]	155 [107, 229]	133 [96, 219]
Creatinine (mg/dL)	1.8(0.6)	2.4 (0.7)	1.9 (0.6)	1.5 (0.3)	1.3 (0.3)
Cystatin C (mg/L)	1.5 (0.4)	2.2 (0.3)	1.6 (0.1)	1.2 (0.1)	1.0 (0.1)
B2M (mg/L)	3.8 (1.8)	6.5 (2.1)	3.8 (1.0)	2.8 (0.6)	2.2 (0.4)
$eGFR_{cr}$ (mL/min/1.73m^2)	46.0 (18.2)	30.7 (12.3)	41.4 (13.2)	54.8 (15.2)	62.8 (19.4)
$eGFR_{cys}$ (mL/min/1.73m^2)	43.8 (15.0)	26.1 (5.9)	38.4 (7.1)	52.5 (7.7)	65.5 (11.5)
$eGFR_{B2M}$ (mL/min/1.73m^2)	48.8 (16.4)	28.8 (6.9)	44.4 (8.9)	57.2 (9.9)	71.1 (12.8)
Urine Albumin to Creatinine Ratio (mg/g)	25 [10, 110]	64 [18, 435]	29 [10, 112]	15 [7, 47]	16 [8, 58]

Note: Continuous variables are presented as mean (standard deviation) or as median [25th percentile, 75th percentile].

Table 2

Association of filtration markers with risk of adjudicated cardiovascular events (N=306 events) in stable kidney transplant recipients in FAVORIT

	Demographic and Transplant Characteristic Adjusted*		Multivariable Adjusted^		Multivariable Adjusted^ + $eGFR_{cr}$	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
$eGFR_{cys}$						
<30	3.58 (2.08, 6.15)	<0.001	2.02 (1.09, 3.76)	0.03	3.05 (1.41, 6.57)	0.005
30 to <45	1.78 (1.07, 2.97)	0.03	1.56 (0.89, 2.77)	0.13	2.09 (1.07, 4.06)	0.03
45 to <60	1.32 (0.75, 2.29)	0.33	0.93 (0.49, 1.79)	0.83	1.04 (0.54, 2.02)	0.90
60+	Reference	–	Reference	–	Reference	–
	p-trend	<0.001	p-trend	0.03	p-trend	0.008
per 10 mL/min/1.73m^2	0.72 (0.63, 0.82)	<0.001	0.78 (0.68, 0.90)	<0.001	0.68 (0.56, 0.83)	<0.001
$eGFR_{B2M}$						
<30	3.66 (2.19, 6.13)	<0.001	2.56 (1.35, 4.88)	0.004	3.16 (1.54, 6.50)	0.002
30 to <45	2.10 (1.34, 3.29)	0.001	1.71 (0.96, 3.03)	0.07	1.99 (1.06, 3.74)	0.03
45 to <60	1.39 (0.87, 2.23)	0.17	1.16 (0.64, 2.08)	0.63	1.24 (0.68, 2.24)	0.48
60+	Reference	–	Reference	–	Reference	–
	p-trend	<0.001	p-trend	0.01	p-trend	0.006
per 10 mL/min/1.73m^2	0.75 (0.67, 0.83)	<0.001	0.80 (0.70, 0.91)	<0.001	0.74 (0.63, 0.87)	0.003
$eGFR_{cr}$						
<30	1.26 (0.78, 2.05)	0.34	1.16 (0.66, 2.03)	0.61	–	–
30 to <45	1.51 (0.97, 2.34)	0.07	1.29 (0.76, 2.18)	0.34	–	–
45 to <60	0.84 (0.52, 1.34)	0.45	0.83 (0.48, 1.44)	0.51	–	–
60+	Reference	–	Reference	–	–	–
	p-trend	0.02	p-trend	0.32	–	–
per 10 mL/min/1.73m^2	0.94 (0.86, 1.03)	0.20	0.96 (0.88, 1.06)	0.46	–	–

Note: Weighted Cox regression models were performed in the cardiovascular events analytic sample of 760 participants with 306 events. Results in the shaded area are not presented because models with eGFRcr as the primary exposure cannot be additionally adjusted for eGFRcr.

*Adjusted for age, sex, race, country, randomized treatment assignment, donor type, graft vintage

^ Adjusted for age, sex, race, country, randomized treatment assignment, donor type, graft vintage, prevalent cardiovascular disease, diabetes, smoking status, LDL cholesterol, HDL cholesterol, triglycerides, systolic blood pressure, diastolic blood pressure, body mass index, and natural log transformed urinary albumin to creatinine ratio.

Table 3

Association of filtration markers with risk of all-cause mortality (N=382 events) in stable kidney transplant recipients in FAVORIT

	Demographic and Transplant Characteristic Adjusted*		Multivariable Adjusted^		Multivariable Adjusted^ + $eGFR_{cr}$	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
$eGFR_{cys}$						
<30	5.88 (3.35, 10.34)	<0.001	3.92 (2.11, 7.31)	<0.001	8.55 (3.84, 19.03)	<0.001
30 to <45	2.03 (1.18, 3.49)	0.01	1.76 (0.97, 3.21)	0.06	3.10 (1.52, 6.33)	0.002
45 to <60	1.59 (0.87, 2.88)	0.13	1.33 (0.67, 2.63)	0.42	1.75 (0.86, 3.57)	0.13
60+	Reference	–	Reference	–	Reference	–
	p-trend	<0.001	p-trend	<0.001	p-trend	<0.001
per 10 mL/min/1.73m^2	0.62 (0.54, 0.71)	<0.001	0.67 (0.58, 0.78)	<0.001	0.51 (0.41, 0.63)	<0.001
$eGFR_{B2M}$						
<30	5.53 (3.33, 9.20)	<0.001	4.09 (2.21, 7.54)	<0.001	6.10 (3.06, 12.19)	<0.001
30 to <45	2.56 (1.62, 4.04)	<0.001	2.38 (1.37, 4.13)	0.002	3.28 (1.79, 6.02)	0.001
45 to <60	1.53 (0.95, 2.47)	0.08	1.37 (0.77, 2.45)	0.28	1.62 (0.91, 2.90)	0.10
60+	Reference	–	Reference	–	Reference	–
	p-trend	<0.001	p-trend	<0.001	p-trend	<0.001
per 10 mL/min/1.73m^2	0.66 (0.59, 0.74)	<0.001	0.69 (0.61, 0.79)	<0.001	0.61 (0.52, 0.71)	<0.001
$eGFR_{cr}$						
<30	1.23 (0.76, 1.99)	0.40	1.09 (0.63, 1.87)	0.76	–	–
30 to <45	1.37 (0.89, 2.12)	0.15	1.21 (0.74, 1.98)	0.49	–	–
45 to <60	0.99 (0.63, 1.56)	0.98	1.03 (0.62, 1.72)	0.90	–	–
60+	Reference	–	Reference	–	–	–
	p-trend	0.30	p-trend	0.85	–	–
per 10 mL/min/1.73m^2	0.95 (0.88, 1.04)	0.28	0.97 (0.88, 1.06)	0.49	–	–

Note: Weighted Cox regression models were performed in the all-cause mortality analytic sample of 822 participants with 382 events. Results in the shaded area are not presented because models with eGFRcr as the primary exposure cannot be additionally adjusted for eGFRcr.

*Adjusted for age, sex, race, country, randomized treatment assignment, donor type, graft vintage

^ Adjusted for age, sex, race, country, randomized treatment assignment, donor type, graft vintage, prevalent cardiovascular disease, diabetes, smoking status, LDL cholesterol, HDL cholesterol, triglycerides, systolic blood pressure, diastolic blood pressure, body mass index, and natural log transformed urinary albumin to creatinine ratio.

Table 4

Association of filtration markers with risk of dialysis-dependent kidney failure (n=280 events) in stable kidney transplant recipients in FAVORIT

	Demographic and Transplant Characteristic Adjusted*		Multivariable Adjusted^		Multivariable Adjusted^ + $eGFR_{cr}$	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
$eGFR_{cys}$						
<30	15.90 (7.73, 32.68)	<0.001	9.49 (4.28, 21.00)	<0.001	12.74 (4.88, 33.29)	<0.001
30 to <45	3.76 (1.88, 7.52)	<0.001	2.68 (1.20, 5.96)	0.02	3.24 (1.36, 7.71)	0.008
45 to <60	2.24 (1.05, 4.78)	0.04	1.98 (0.79, 4.92)	0.14	2.18 (0.88, 5.39)	0.09
60+	Reference	–	Reference	–	Reference	–
	p-trend	<0.001	p-trend	<0.001	p-trend	<0.001
per 10 mL/min/1.73m^2	0.45 (0.37, 0.55)	<0.001	0.50 (0.39, 0.62)	<0.001	0.42 (0.32, 0.56)	<0.001
$eGFR_{B2M}$						
<30	22.67 (11.78, 43.63)	<0.001	15.53 (6.99, 34.51)	<0.001	18.62 (7.64, 45.39)	<0.001
30 to <45	5.76 (3.17, 10.48)	<0.001	4.59 (2.14, 9.83)	<0.001	5.24 (2.29, 12.00)	<0.001
45 to <60	3.10 (1.66, 5.82)	<0.001	2.56 (1.18, 5.58)	0.02	2.73 (1.24, 6.01)	0.01
60+	Reference	–	Reference	–	Reference	–
	p-trend	<0.001	p-trend	<0.001	p-trend	<0.001
per 10 mL/min/1.73m^2	0.51 (0.44, 0.59)	<0.001	0.53 (0.44, 0.64)	<0.001	0.49 (0.39, 0.61)	<0.001
$eGFR_{cr}$						
<30	3.13 (1.85, 5.28)	<0.001	2.09 (1.05, 4.14)	0.04	–	–
30 to <45	2.01 (1.22, 3.33)	0.007	1.72 (0.90, 3.27)	0.10	–	–
45 to <60	0.76 (0.43, 1.33)	0.33	0.65 (0.31, 1.36)	0.25	–	–
60+	Reference	–	Reference	–	–	–
	p-trend	<0.001	p-trend	0.001	–	–
per 10 mL/min/1.73m^2	0.74 (0.66, 0.83)	<0.001	0.80 (0.70, 0.92)	0.002	–	–

Note: Weighted Cox regression models were performed in the dialysis dependent kidney failure analytic sample of 736 participants with 280 events. Results in the shaded area are not presented because models with eGFRcr as the primary exposure cannot be additionally adjusted for eGFRcr.

*Adjusted for age, sex, race, country, randomized treatment assignment, donor type, graft vintage

^ Adjusted for age, sex, race, country, randomized treatment assignment, donor type, graft vintage, prevalent cardiovascular disease, diabetes, smoking status, LDL cholesterol, HDL cholesterol, triglycerides, systolic blood pressure, diastolic blood pressure, body mass index, and natural log transformed urinary albumin to creatinine ratio