Supplemental Material: Bajaj NS et al. Coronary microvascular dysfunction, left ventricular remodeling and clinical outcomes in patients with chronic kidney impairment.

Contents

Supplemental references

Supplemental Methods

Clinical outcomes

A Clinical Endpoints Committee (CEC) reviewed and adjudicated the primary composite endpoint (all cause deaths, non-fatal MI, and hospitalizations for heart failure). The members of CEC were blinded to image analyses. Time to first event defined as a composite of all cause death, and hospitalization for nonfatal MI or heart failure was analyzed. Ascertainment of clinical endpoints were determined by CEC from the longitudinal medical record, Partners Healthcare Research Patient Data Registry (RPDR), the National Death Index, mail surveys and telephone calls. Follow up was censored on December 31st, 2017. The RPDR is a centralized clinical data registry, or data warehouse, that gathers clinical information from various Partners hospital systems, the details can be found at https://rc.partners.org/research-apps-and-services/identify-subjects-request-data#research-patient-data-registry.

The definitions of individual outcomes are as below:

- Deaths: Ascertained from death certification, medical records, mail surveys and telephone calls.

- Non-fatal MI: 2012 Third Universal Definition of Myocardial Infarction was used to define Myocardial infarction.[1]

- Hospitalizations for heart failure (HF): Defined as an admission was defined by an event meeting all of the following criteria: admission to an inpatient unit or emergency department for at least 12 hours with a discharge diagnosis consistent with HF, at least one clinical manifestation of HF (i.e. dyspnea, orthopnea, paroxysmal nocturnal dyspnea, edema, pulmonary crackles or radiographic evidence of worsening HF), and additional or increased therapy for HF (initiation or increase of intravenous diuretics, inotropes or vasodilators or initiation of a medical or surgical intervention aimed at treating HF).

Statistical Analysis

Stata software version 15.1 (StataCorp, College Station, Texas) and R (version 3.6.0) were used for analyses.

Baseline characteristic comparisons

Differences in baseline characteristics among those with eGFR $\geq$ 60 versus those with eGFR< 60 were compared by $\chi 2$ tests for categorical and Mann-Whitney U test for continuous variables.

Modelling for myocardial function and outcomes

Diastolic indices

Unadjusted and multivariable-adjusted associations between eGFR & CFR, diastolic indices (Lateral E/e' and Septal E/e') were evaluated using linear regression models using restricted cubic splines. N=279 had information of Lateral E/e' and N=287 had information on Medial E/e'. The multivariable models included demographic factors (age, sex, race) and clinical factors (history of hypertension, diabetes, peripheral vascular disease, stroke, diabetes, eGFR, CFR, body mass index (BMI), LVMI and LVEF). The variables and number of knots were selected based on optimal values of the Akaike information criterion after including clinically important covariates. Three equally spaced knots were optimal for CFR and eGFR. The 3-dimesional scatter and surface plots were plots were drawn using CAR and RGL package in R version 3.6.0. Those with missing data were excluded from fully adjusted models. The p-values from likelihood chi square test of significance of spline terms in the multivariate model were reported as p-trend. Correlation and partial correlations were also calculated.

Systolic indices

Unadjusted and multivariable-adjusted associations between eGFR & CFR, systolic indices (GLS, GRS and GCS) were evaluated using linear regression models using restricted cubic splines. GLS was available on 349, GRS on 287 and GCS on 290 patients respectively. The multivariable models included demographic factors (age, sex, race) and clinical factors (history of hypertension, diabetes, peripheral vascular disease, stroke, diabetes, eGFR, CFR, body mass index (BMI), LVMI and LVEF). The variables and number of knots were selected based on optimal values of the Akaike information criterion after including clinically important covariates. Four equally spaced knots were optimal for CFR and three for eGFR. The 3-dimesional scatter and surface plots were plots

were drawn using CAR and RGL package in R version 3.6.0. Those with missing data were excluded from fully adjusted models. The p-values from likelihood chi square test of significance of spline terms in the multivariate model were reported as p-trend. Correlation and partial correlations were also calculated.

LV wall stress

Unadjusted and multivariable-adjusted associations between eGFR & CFR, circulating marker of LV wall stress (NTproBNP) were evaluated using linear regression models using restricted cubic splines. NTproBNP levels were available on 82 patients. NTproBNP levels were natural log transformed for this analysis. The multivariable models included demographic factors (age, sex, race) and clinical factors (history of hypertension, diabetes, peripheral vascular disease, stroke, diabetes, eGFR, CFR, body mass index (BMI), LVMI and LVEF). The variables and number of knots were selected based on optimal values of the Akaike information criterion after including clinically important covariates. Three equally spaced knots were optimal for CFR and eGFR. The 3-dimesional scatter and surface plots were plots were drawn using CAR and RGL package in R version 3.6.0. Those with missing data were excluded from fully adjusted models. The p-values from likelihood chi square test of significance of spline terms in the multivariate model were reported as p-trend. Correlation and partial correlations were also calculated.

Adverse composite clinical endpoint (MACE)

Unadjusted and multivariable-adjusted associations between eGFR & CFR, and adverse composite clinical endpoint were evaluated using Poisson and Cox proportional hazard models while accounting for non-linearity of relationships using restricted cubic splines. The multivariable models included demographic factors (age, sex, race) and clinical factors (history of hypertension, diabetes, peripheral vascular disease, stroke, diabetes, eGFR, CFR, body mass index (BMI), LVMI and LVEF). We also tested for a statistical interaction between CFR and eGFR in the multivariate model. The variables and number of knots were selected based on optimal values of the Akaike information criterion after including clinically important covariates. Three equally spaced knots were optimal for CFR and eGFR. Those with missing data were excluded from fully adjusted models. The p-values from likelihood chi square test of significance of spline terms in the multivariate model were reported as p-trend.

Cox proportional hazard modelling to understand the effect of abnormal eGFR and CFR

Unadjusted and multivariable-adjusted Cox proportional hazards models were used to determine the effect of abnormal eGFR[2] (<60 ml/min/1.73m^2) and severely abnormal CFR (<1.5). The multivariable models included demographic factors (age, sex, race) and clinical factors (history of hypertension, diabetes, peripheral vascular disease, stroke, diabetes, eGFR, CFR, body mass index (BMI), LVMI and LVEF). The cox proportional hazard (PH) assumptions test based on Schoenfeld residuals was used to verify non-violation of PH assumption in the adjusted model. We imputed values of LVMI and BMI using regression/conditional mean imputation and reanalyzed the data.

Interplay between LV structure, myocardial mechanics and coronary microvascular dysfunction on future outcomes

To understand the interplay between LV structure, myocardial mechanics and coronary microvascular dysfunction on future outcomes, we performed an exploratory analysis where we stratified patients by abnormal geometry, diastolic dysfunction (E/e'<15), systolic dysfunction (GLS<-17%) and CFR (<1.5) and compared the rate of adverse events using Poisson regression as well as a composite of heart failure admissions and non-fatal MI. The cutoffs for GLS were at median values in our cohort, whereas ASE definitions were used to define abnormal geometry[3], cutoffs for E/e' and CFR were based on previous studies.[4, 5] The groups compared were abnormal CFR and Abnormal geometry vs others, abnormal CFR and E/e'>15 vs others and abnormal CFR and GLS<-17% vs. others. The p-values are from adjusted Poisson models.

Mediation Analysis

Mediation analysis-which tests a putative causal relation among variables (i.e., a path)—was also performed to test whether renal function exerts its effect on cardiovascular disease via microvascular dysfunction. eGFR was chosen as measure of renal function

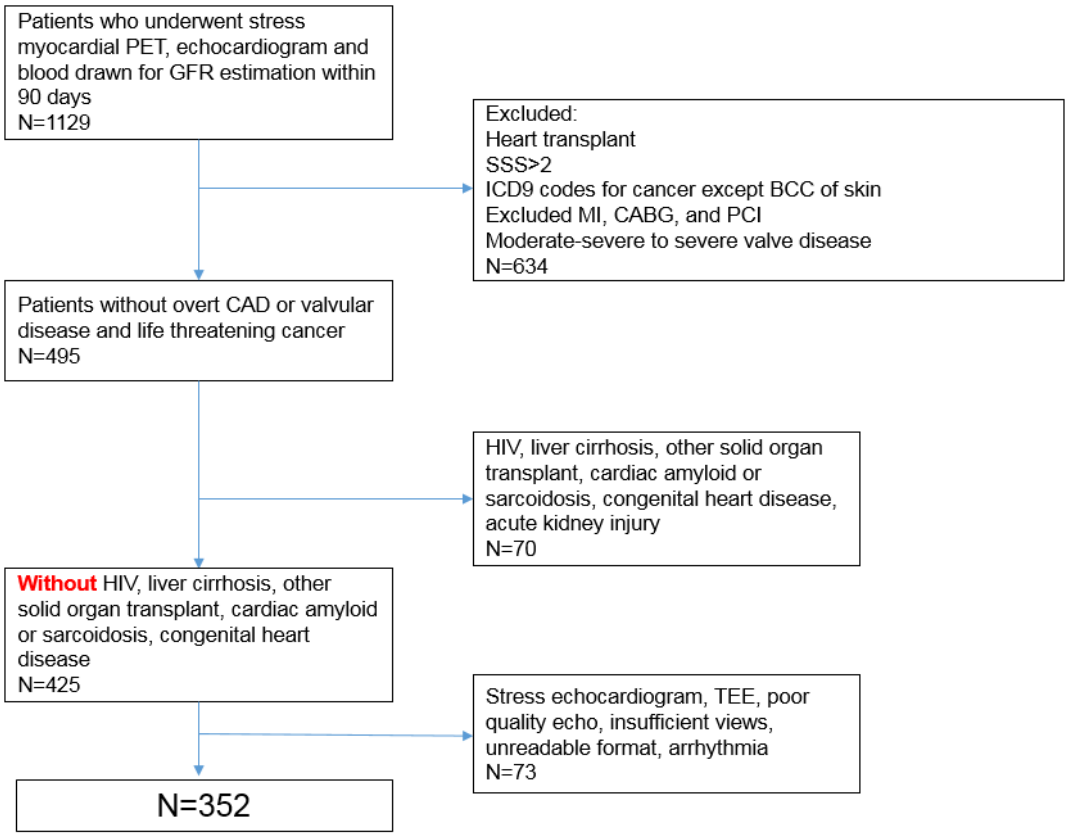
(exposure), CFR was chosen as marker of microvascular disease (mediator) whereas measures of diastolic/systolic function, NTproBNP and clinical composite endpoint were chosen as markers of cardiovascular disease (outcome).[6] All analyses were adjusted for demographic factors (age, sex, race) and clinical factors (history of hypertension, diabetes, peripheral vascular disease, stroke, diabetes, eGFR, CFR, body mass index (BMI), LVMI and LVEF). Linear regression and logistic regression models were used for continuous (E/e', GLS, GCS, radial strain and NTproBNP) and categorical outcomes(MACE) respectively.

We used medeff package in STATA to perform all analysis[6]. The following steps were followed:

Step 1: We fit models for the observed outcomes (measures of diastolic, systolic, LV wall stress and composite endpoint) mediator variable (CFR) and covariates (as above).
Step 2: We simulated model parameters from their sampling distribution (1000 simulations were performed).
Step 3: Repeated the following three steps for each draw of model parameters:
1. Simulated the potential values of the mediator.
2. Simulated the potential outcomes given the simulated values of the mediator.
3. Computed quantities of interest (Average causal mediated effect (ACME), Average direct effect (ADE), and average total effect).
Step 4: Computed summary statistics, i.e. point estimates (average) and confidence intervals.

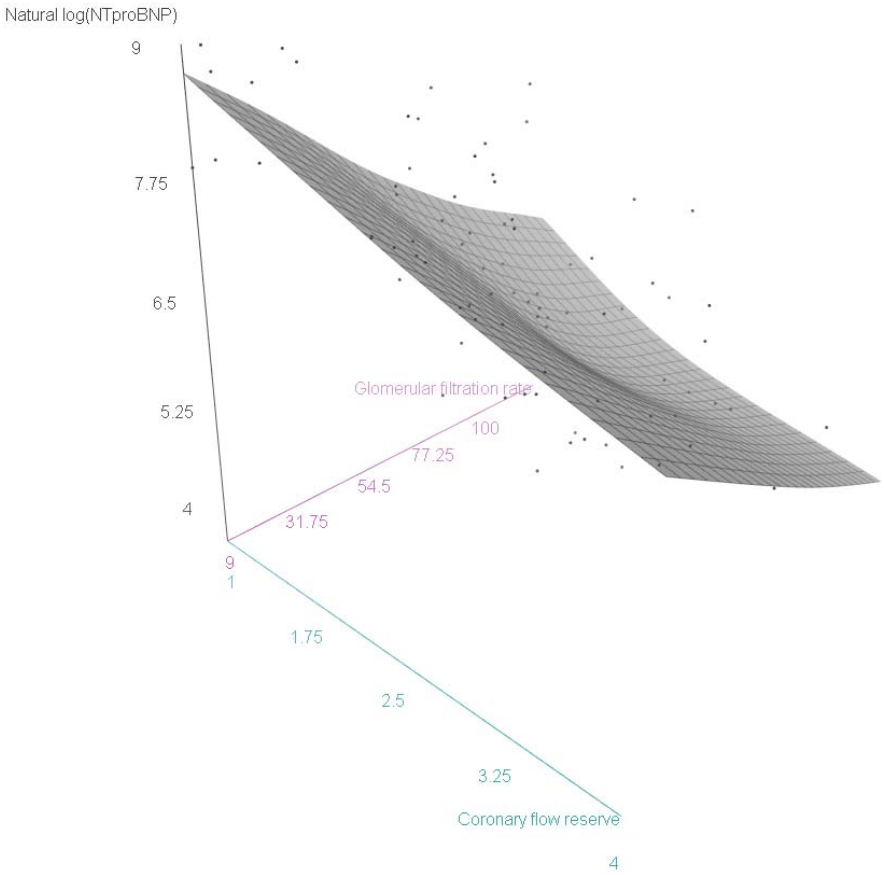
Medeff package doesn't allow implementation of Cox model for mediation analysis hence for MACE outcome we used a med4way package in STATA[7] which allows for implementation of Cox model was for mediation analysis. The results for mediation analysis using both Cox and Logistic model are presented below in **Supplemental Table 6**.

Supplementary results



Supplemental Figure 1: Flow diagram describing selection of study cohort

PET= Positron emission tomography, SSS= summed stress score, ICD= International Classification of Diseases 9th Revision, BCC: Basal Cell Carcinoma, MI= myocardial infarction, PCI= percutaneous coronary intervention, CABG= Coronary artery bypass grafting, CAD= coronary artery disease, HIV= Human immunodeficiency virus, TEE= trans-esophageal echocardiogram



Supplemental Figure 2: Relationship between Natural LogNTproBNP, CFR and GFR using a restricted cubic spline linear regression (**Black grid on gray surface**).

Adjusted regression models included CFR=Coronary Flow Reserve, eGFR= estimated Glomerular Filtration, Age, Gender, Race, Hypertension, Hyperlipidemia, Diabetes, Peripheral vascular disease, Stroke, Left ventricular mass indexed and resting left ventricular ejection fraction

NTproBNP= N-terminal pro B-type natriuretic peptide

Supplemental Table 1: Intraobserver and interobserver intraclass correlations for echocardiographic measurements.

Echocardiographic Measurement	Intraclass correlation coefficients	
	Intra-observer (N=10)	Inter-observer (N=10)
LVEF	0.93	0.84
E velocity	0.99	0.97
Lateral e’	0.82	0.92
Septal e’	0.86	0.82
GLS	0.94	0.93

Two readers and 10 targets.

Supplemental Table 2: STROBE Statement—checklist of items that should be included in reports of observational studies				
	Item No.	**Recommendation**	**Page No.**	**Relevant text from manuscript**
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3	Abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3	Abstract
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5	Introduction
Objectives	3	State specific objectives, including any prespecified hypotheses	5	Introduction
Methods				
Study design	4	Present key elements of study design early in the paper	5-10, Supplement	Methods
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-10	Methods
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5-10, Supplemental Figure 1	Methods
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA	

Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8 and Supplemetal methods	Methods
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-10*	*Methods*
Bias	9	Describe any efforts to address potential sources of bias	5-10	Methods
Study size	10	Explain how the study size was arrived at	Supplemental Figure 1	Methods and supplement

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-10	Methods
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5-10 and Supplemental methods	Methods and Suppelement
		(*b*) Describe any methods used to examine subgroups and interactions	5-10 and Supplemental methods	Methods and Suppelement
		(*c*) Explain how missing data were addressed	5-10 and Supplemental methods	Methods and Supplement
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	5-10 and Supplemental methods	Methods and Supplement
		(*e*) Describe any sensitivity analyses	5-10 Methods and Supplement	Methods and Supplement
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Supplemental Figure 1	Supplement
		(b) Give reasons for non-participation at each stage	Supplemental Figure 1	Supplement
		(c) Consider use of a flow diagram	Suplemetal Figure 1	Supplement
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1	Results
		(b) Indicate number of participants with missing data for each variable of interest	Supplemental Table 5	Supplement

		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Table 2	Results
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Table 2	Results
		Case-control study—Report numbers in each exposure category, or summary measures of exposure		
		Cross-sectional study—Report numbers of outcome events or summary measures		
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5-10	Methods and Results
		(*b*) Report category boundaries when continuous variables were categorized	10-13	Results
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10-13 and Tables	Results

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10-13	Results
Discussion				
Key results	18	Summarise key results with reference to study objectives	13-15	Discussion
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13-15	Discussion
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-15	Disccusion
Generalisability	21	Discuss the generalisability (external validity) of the study results	13-15	Disccusion
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	2-4	Title and Abstarct

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Supplemental Table 3: Association between eGFR and CFR in models without and with CCB use			
	Correlation coefficient for eGFR and CFR association	Unadjusted p-value in model	Adjusted p-value
Model 1 (Unadjusted association)	0.2604	<0.001	-
Model 2 (Model 1 + CCB Use)	0.2596*	-	<0.001
Model 3 (Model 1 + Confounders)	0.1205*	-	0.0317
Model 4 (Model 1 + Confounders+ CCB use)	0.1258*	-	0.0251

Confounders included: age, gender, race, hypertension, hyperlipidemia, diabetes, peripheral vascular disease, stroke, left ventricular mass indexed and left ventricular ejection fraction. *Partial correlation coefficients after accounting for confounders.

Supplemental Table 4: Associations between LV mechanics, renal function and coronary flow reserve in diabetics								
Measure	**Association with CFR**				**Association with eGFR**			
	Shape	Correlation Coefficient	Unadjusted,p-value	Adjusted, p-value	Shape	Correlation Coefficient	Unadjusted, p-value	Adjusted, p-value
Diastolic indices								
Lateral E/e’	L-shaped	-0.38	<0.001	0.005	L-shaped	-0.34	0.001	0.251
Septal E/e’	L-shaped	-0.39	<0.001	0.017	L-shaped	-0.29	0.002	0.063
Systolic indices								
GLS	Reverse sigmoid	-0.57	<0.001	0.001	Linear	-0.30	0.001	0.162
GRS	Sigmoid	0.29	<0.001	0.001	Linear	0.20	0.002	0.250
GCS	Reverse sigmoid	-0.42	<0.001	0.001	Linear	-0.29	0.001	0.584

Adjusted regression models included CFR, eGFR, age, gender, race, hypertension, hyperlipidemia, peripheral vascular disease, stroke, left ventricular mass indexed and left ventricular ejection fraction. For continuous outcomes (Lateral E/e’, Septal E/e’, GLS, GRS, GCS and Natural log NTproBNP linear regression restricted spline models were used) whereas for MACE Poisson regression restricted cubic spline models were used)

Supplemental table 5: Comparison of models without and with imputation.			
Current model, N=327		**Imputed data, N=352**	
CFR >=1.5 vs. CFR<1.5			
Unadjusted HR	Adjusted HR	Unadjusted HR	Adjusted HR
2.02 (1.36-2.98)	1.61 (1.05-2.46)	2.13 (1.46-3.11)	1.66 (1.09-2.52)
eGFR >=60 vs. eGFR<60			
Unadjusted HR	Adjusted HR	Unadjusted HR	Adjusted HR
2.43 (1.64-3.31)	1.29 (0.82-2.04)	2.55 (1.73-3.75)	1.36 (0.87-2.10

Supplemental table 6: Frequency of missing data

Variable	Outcome/Dependent variable	Confounder/ Covariate	Mediator	Exposure	Number available
eGFR	No	No	No	Yes	352/352
CFR	No	No	Yes	No	352/352
GLS	Yes	No	No	No	349/352
GRS	Yes	No	No	No	287/352
GCS	Yes	No	No	No	290/352
Lateral E/e’	Yes	No	No	No	279/352
Septal E/e’	Yes	No	No	No	287/352
NTproBNP	Yes	No	No	No	82/352
MACE	Yes	No	No	No	352/352
Age	No	Yes	No	No	352/352
Gender	No	Yes	No	No	352/352
Race	No	Yes	No	No	352/352
Hypertension	No	Yes	No	No	352/352
Hyperlipidemia	No	Yes	No	No	352/352
Diabetes	No	Yes	No	No	352/352
PAD	No	Yes	No	No	352/352
Stroke	No	Yes	No	No	352/352
LVEF	No	Yes	No	No	351/352
LVMI	No	Yes	No	No	328/352
BMI	No	Yes	No	No	350/352

Supplemental Table 7: Mediation analysis (Logistic vs. Cox model)		
Adverse cardiovascular event using Logistic model in mediation analysis		
	Standardized Regression coefficients for CFR in mediation model, p-value	**% effect mediated via CFR**
Composite clinical endpoint (MACE)	-0.32, 0.039	14%
Adverse cardiovascular event using Cox model in mediation analysis		
Composite clinical endpoint (MACE)	-0.30, 0.026	32%

Results for Mediation model for hypothesized pathway via microvascular dysfunction to adverse cardiovascular event in chronic kidney disease

Supplemental References

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