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Inflammation and trajectory of renal function in community-dwelling older adults

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Abstract

BACKGROUND/OBJECTIVES—Limited evidence suggests that the inflammatory state of aging is a risk factor for accelerated renal function (RF) decline. We examined this hypothesis using inflammatory biomarkers and RF collected over a 9-year follow-up in relatively healthy individuals enrolled in the InCHIANTI study.

DESIGN—Longitudinal

SETTING—Community

PARTICIPANTS—Participants included 687 adults age 60 and older with baseline estimated glomerular filtration rate (eGFR) 60 ml/min/1.73m² and no diabetes mellitus (DM).

MEASURES—eGFR, proxy for RF, was determined using the CKD-EPI equation at baseline and then at 3-, 6-, and 9-year follow-ups. Incident chronic kidney disease (CKD) was defined as new-onset eGFR < 60 ml/min/1.73m² at each follow-up. Predictors included baseline and time-dependent inflammatory biomarkers: soluble tumor necrosis factor alpha receptors (sTNFα-R1 and -R2), interleukin(IL)-6, IL-18, IL-1β, IL-1 receptor antagonist, and high sensitivity C-reactive protein.

Results—Higher baseline sTNFα-R1 was significantly associated with lower eGFR over 9 years, independent of DM or blood pressure: (Baseline: $\hat{\beta} = -0.39$, $p = 0.001$; 3-year: $\hat{\beta} = -0.26$, $p = 0.001$; 6-

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Conflict of interest

None.

The results presented in this paper have not been published previously in whole or part, except in abstract format.

Author Contributions

All authors contributed to this paper.

Sponsor's Role

None.

year: $\hat{\beta} = -0.36$, $p = 0.001$; 9-year: $\hat{\beta} = -0.47$, $p = 0.001$). The rate of TNF α -R1 change was significantly associated with rate of eGFR change ($\hat{\beta} = -0.18$, $p = 0.001$). Baseline sTNF α -R1 predicted incident CKD [per 1-SD increment]: 3-year: relative risk (RR)=1.3 (95% CI: 1.1–1.5); 6-year: RR=1.5 (1.1–2.2); 9-year RR=1.6 (1.1–2.2.). Similar results were found for sTNF α -R2.

Conclusion—Baseline and the rate of change in TNF α receptors were significantly associated with faster RF decline and incident CKD among older adults independent of DM or blood pressure.

Keywords

Cytokines; inflammation; renal function; CKD; aging

Introduction

Chronic kidney disease (CKD) is a major health burden worldwide.¹ Diabetes mellitus (DM) and essential hypertension (HTN) are established risk factors for CKD,² yet they do not fully explain the increased risk of renal function (RF) decline and CKD with aging.^{3, 4} The role of high concentrations of pro-inflammatory cytokines has drawn considerable attention because studies have shown that patients with known CKD and high inflammation experience accelerated declines in RF.^{5, 6} RF also declines with aging in the absence of major comorbidities.^{7–10} Moreover, chronic inflammation is known as a feature of aging.¹¹ There is body of evidence showing associations between inflammatory biomarkers, which activate two main inflammatory pathways, and age-related diseases such as functional decline, mobility and cognitive impairments, and mortality.^{11–14} Examples of such biomarkers include interleukin-6 (IL-6) and tumor necrosis factor α (TNF α) that activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), and IL-1 β and IL-18 that are related to the nucleotide-binding oligomerization domain (NOD)-like receptor containing pyrin domain 3 (NLRP3) pathways.

To date, the results of studies that show inflammation predicts incident CKD are still controversial because of methodological limitations, including limited number of inflammatory biomarkers, not considering potentially informative censoring and loss to follow-up, or limited follow-up time.^{14–18} Moreover, most of these studies were focused on patients with DM. In this study we focused on IL-6 and two soluble TNF α receptors (sTNF α -R1 and sTNF α -R2) from NF- κ B pathway, IL-18 and IL-1 β from NLRP3 pathway, IL-1 receptor antagonist, and HS-CRP.¹⁴

Using InCHIANTI data,¹⁹ we tested if baseline levels of these inflammatory biomarkers and their increased levels over 9 years were prospectively associated with accelerated RF decline and CKD development in nondiabetic participants who initially had normal RF.

Methods

Study Design and Setting

Analyses were performed using 9 years of prospective data from the InCHIANTI (*Invecchiare in Chianti*) study. Participants were enrolled from 1998–2000 and had follow-

up visits every 3 years. The 3-year follow-up occurred in 2001–2003, the 6-year in 2004–2006, and the 9-year in 2007–2009. All participants explicitly consented that their collected data could be used for research purposes.

Participants

Of 1,453 individuals, 1,326 had measured baseline serum creatinine. Exclusion criteria for these analyses included age < 60 years (n = 237); baseline estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73m² (n = 232); DM defined as fasting plasma glucose ≥ 126 mg/dL, history of DM or antidiabetic medication (n = 117); history of steroid utilization (n = 15); high sensitive C-reactive protein (HS-CRP) ≥ 30 g/L (n = 9) to exclude persons with severe infections or autoimmune diseases that resulted in exclusion of the outliers of other biomarkers as well; and missing baseline TNFα-R1 (n = 29). Because some individuals met multiple exclusion criteria, the baseline sample size was 687.

Measurements

Inflammatory biomarkers

Measured serum levels of inflammatory biomarkers included sTNFα-R1, sTNFα-R2, IL-18, and IL-6 at baseline and all three follow-ups, while IL-1 receptor antagonist (IL-1RA) and IL-1β were measured only at baseline, using previously described assays.^{20, 21} IL-1β and IL-1RA were measured in duplicate using high-sensitivity enzyme-linked immunoabsorbent assays (ELISA) (BIOSOURCE International, Camarillo, CA). IL-18 was measured using highly sensitive quantitative sandwich assays (Quantikine HS, R&D Systems, Minneapolis, MN). sTNFα-R1 and sTNFα-R2 were measured utilizing ELISA (BIOSOURCE International, Camarillo, CA). HS-CRP was measured using colorimetric competitive immunoassay with purified protein and polyclonal anti-CRP antibodies (Calbiochem, San Diego, CA). We calculated the inter-assay and intra-assay coefficient of variation (CV) at baseline and all follow-ups (Table S1). Additionally, to understand “within-subject” variations of the inflammatory biomarkers in the total study population and in those who never developed CKD, we performed a mixed-effect model for each longitudinal biomarker over 9-year follow-ups and reported random estimated within-subject variations with 95% confidence interval (95% CI) (Table S2). From InCHIANTI genotyped data we also extracted a genetic variant for R92Q mutation (rs4149584) within the tumor necrosis factor receptor superfamily member 1A (*TNFRSF1A*), a gene that encodes TNFα-R1,²² as this variant increases the affinity of TNFα to TNFα-R1.²³

Covariates

Demographic covariates included age; sex; smoking, reported as “never,” “former,” or “current”; marital status, reported as “not married,” “married,” or “widowed,”; and education (number of school years). Systolic blood pressure (SBP), measured three times in both arms, was then determined as the average of the three maximum measures. Similarly, diastolic blood pressure (DBP) was determined. Use of angiotensin converting enzyme inhibitor (ACEI), angiotensin II receptor blockers (ARBs), or nonsteroidal anti-inflammatory drugs and presence of cancer were self-reported. We adjusted for new-onset DM over the time course. A diagnosis of congestive heart failure (CHF) was adjudicated

based on self-reported history and/or current treatment for CHF. Baseline urinary protein (normal range for proteinuria was 0–150mg/dL)²⁴ was measured using a Modular P800 Hitachi Analyzer and normalized to urine creatinine concentration, and the details of urine creatinine measure is described elsewhere.^{25, 26} Creatinine levels depend on muscle mass, which often declines with aging; therefore, we included calf muscle cross-sectional area (proxy for muscle mass) derived from computerized tomography and changes in cross-sectional area over time.²⁷

Outcomes

Serum creatinine was quantified using an enzymatic assay and calibrated to standardized isotope dilution mass spectrometry (IDMS).²⁸ Glomerular filtration rate (eGFR), a proxy for RF, was estimated from IDSM-traceable serum creatinine, age, and sex using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (henceforth called eGFR).^{29, 30} We also used the rate of eGFR change over 9 years as an outcome (slope, see Statistical Analysis). Moreover, we defined incident CKD as the development of eGFR<60 ml/min/1.73m² in individuals who had normal eGFR (≥60 ml/min/1.73m²) at the previous visit.

Statistical analyses

Participants' characteristics are reported as means±standard deviation (SD) or median and interquartile range (IQR), percentages of total study population, and by incident CKD status (Table 1).

The skewed inflammatory biomarkers were natural log-transformed. Then, the biomarkers, SBP, muscle cross-sectional area, and eGFR were standardized to mean =0 and SD=1 to permit comparison of their incremental predictive associations. We used inverse probability weighted generalized estimating equations (W-GEE) to address data missing at random, after accounting for measured covariates and truncation by death.³¹ We assessed the relationship between standardized baseline inflammatory biomarkers and standardized eGFR by separate W-GEE models adjusted for muscle cross-sectional area, SBP, new-onset DM, and other covariates. Results are regression coefficients relating baseline biomarkers with eGFR at each follow-up. To assess which inflammatory biomarker had a stronger association with time-dependent eGFR or rate of change of eGFR over time, we—after evaluating collinearity (Pearson correlation, data not shown)—included all significant biomarkers in a single model adjusting for demographics, CHF, SBP, new-onset DM, and other covariates (see Methods). Then, a parsimonious model was generated by applying backward stepwise elimination of variables with *p*-value>0.1. We also examined possible threshold effects by comparing quartiles 2, 3, and 4 against quartile 1 (reference group).

We computed the rates of change inflammatory biomarkers over time using mixed-effect models and operationalized time as a continuous variable obtaining individual time-slopes as annual rate of change. Similarly, we modeled eGFR change using a mixed-effect model and operationalized time as a continuous variable to obtain annual rate of eGFR (slope), a common RF outcome used in longitudinal studies. We reported the mean±SD of slope in incident CKD versus non-CKD. We determined if baseline inflammatory biomarkers or their

standardized slopes predicted the rate of RF change after accounting for time varying conventional risk factors (SBP and DM) and baseline RF. We adjusted for baseline eGFR to account for inter-individual variability.

The slopes of biomarkers and eGFR were normally distributed. We used standardized slopes (using Z-transformed slopes) for all continuous measures in the models.

The frequently-used single measure of eGFR<60 was considered as an operational definition of CKD. For individuals who had eGFR<60 at one visit and eGFR ≥60 at the next visit, we allowed them to re-enter the analyses in the next visit to calculate incidence rate for the subsequent round of analysis. Therefore, we treated CKD as a recurrent event. We then examined if the inflammatory biomarkers significantly predicted incident CKD within the subsequent follow-up visits using weighted generalized linear models (Poisson distribution model, lag-time) accounting for missing values, with results expressed as relative risk (RR) and 95%CI. All models accounted for covariates described above. We performed all statistical analysis in STATA 14.1(College Station, Texas 77845 USA).

Results

Participant characteristics

Baseline characteristics of the 687 participants are summarized in Table 1. The mean age was 72.9 years (range 60–102 years) and 54% were women. Particularly, participants who experienced incident CKD were older, more likely to be widowed, and had higher baseline levels of SBP, DBP, sTNFα-R1, sTNFα-R2, IL-6, and HS-CRP than participants who did not experience CKD ($p<0.05$).

There were less within-subject variations in estimated inflammatory biomarkers in those with no CKD experience versus those with incident CKD (Table S2). The CKD participants started with lower baseline eGFR than participants who did not experience incident CKD ($p=0.001$). Mean urine protein to creatinine ratio at baseline was 7.3 ± 1.9 mg/g. Mean eGFR at baseline was 71.8 ± 8.9 ml/min/1.73m². The mean eGFR over 9 years for CKD was 51.9 ± 6.6 , but 77.2 ± 9.7 ml/min/1.73m² in non-CKD groups. The mean annual rate of change was -0.09 ± 0.13 ml/min/1.73m² in all participants, while it was -7.6 ± 0.3 in CKD and 1.2 ± 0.1 ml/min/1.73m² in non-CKD groups ($p=0.001$). Further characteristics are shown in Table 1. Only two individuals developed severe loss of kidney function (eGFR 15–29ml/min/1.73m²) and 23 developed new onset DM over the 9-year follow-up. No participant carried a *TNFRSF1A* R92Q variant.

Baseline inflammatory biomarker and RF over time

In separate analyses, after adjustment for covariates, we found that higher baseline serum levels of sTNFα-R1 and TNFα-R2 were associated with significantly lower baseline eGFR ($\hat{\beta}=-0.34$, $p<0.001$; $\hat{\beta}=-0.23$, $p=0.001$, respectively; Figure 1). The association between baseline levels of sTNFα-R1 and lower eGFR remained strongly significant over the 9-year of follow-up ($p<0.001$; Figure 1). A similar association pattern of baseline sTNFα-R2 with lower RF was also observed ($p=0.001$ for all time points; Figure 1).

Other inflammatory biomarkers had less consistent associations with RF. We found that baseline IL-6, IL-1 β , IL-1RA, and IL-18 were not associated with eGFR at baseline ($p>0.05$), but were associated with eGFR at the 6- or 9-year follow-up visits ($p<0.05$; Figure 1). We examined the possibility of a threshold effect by comparing quartiles 2, 3, and 4 against quartile 1 as the reference group. Compared with the first quartile (Q1), participants in the Q2, Q3, and Q4 of baseline sTNF α -R1 and sTNF α -R2 were all significantly associated with steeper declines in eGFR: (sTNF α -R1 Q2: $\hat{\beta} = -0.12$, $p=0.001$; Q3: $\hat{\beta} = -0.20$, $p<0.001$; Q4: $\hat{\beta} = -0.21$, $p=0.001$), and (sTNF α -R2 Q2: $\hat{\beta} = -0.24$, $p=0.001$; Q3: $\hat{\beta} = -0.30$, $p<0.001$; Q4: $\hat{\beta} = -0.28$, $p=0.004$). Similarly, higher baseline TNF α -R1 and TNF α -R2, when considered as a continuous variable (dose-response), were associated with a steeper slope of eGFR decline ($\hat{\beta} = -0.27$, $p=0.001$; $\hat{\beta} = -0.28$, $p=0.001$, respectively). In contrast, the association of baseline IL-6 with the slope of eGFR was not significant when considered as either quartiles (Q2: $\hat{\beta} = -0.03$, $p=0.5$; Q3: $\hat{\beta} = -0.003$, $p=0.8$; Q4: $\hat{\beta} = 0.02$, $p=0.6$) or as a continuous variable. Similar consideration holds for the other nonsignificant biomarkers (results not shown).

In a model that included sTNF α -R1, IL-6, IL-1RA, and HS-CRP, higher levels of baseline sTNF α -R1 remained significantly associated with lower RF over the course of 9-year follow up (3-year follow-up: $\hat{\beta} = -0.25$, $p=0.001$; 6-year follow-up: $\hat{\beta} = -0.37$, $p=0.001$; 9-year follow-up: $\hat{\beta} = -0.45$, $p=0.001$). The associations of baseline IL-6, IL-1RA, and HS-CRP with RF were less consistent than that of sTNF α -R1 with RF (Table S3). In a similar fashion, we replaced sTNF α -R1 with sTNF α -R2, and baseline sTNF α -R2 remained significantly associated with lower eGFR over the course of follow-ups (3-year follow-up: $\hat{\beta} = -0.11$, $p=0.03$; 6-year follow-up: $\hat{\beta} = -0.26$, $p=0.001$; 9-year follow-up: $\hat{\beta} = -0.37$, $p=0.001$). We also found that IL-6, IL-1RA, and HS-CRP remained significant. Other inflammatory biomarkers were not included due to the backward stepwise elimination.

We estimated participant-specific time-slopes of RF (henceforth called “rate” of change; see Methods). In separate W-GEE models, including each baseline biomarker, the eGFR slope was regressed on the baseline biomarkers and adjusted for baseline RF and other covariates. Of note, baseline sTNF α -R1 and IL-1RA were significantly associated with faster declines of RF ($\hat{\beta} = -0.10$, $p<0.001$; $\hat{\beta} = -0.05$, $p=0.001$, respectively; Table S4). Additionally, a sensitivity analysis that removed baseline eGFR from the models resulted in even stronger estimates. In the model evaluating association of baseline TNF α -R1 and slope of eGFR unadjusted for baseline eGFR, the standardized beta coefficient was -0.25 ($p<0.001$).

Rates of inflammatory biomarkers and RF change (slope)

We estimated participant-specific time-slopes of inflammatory biomarkers and RF change and performed separate W-GEE models regressing standardized eGFR slope on the standardized slopes of biomarkers adjusted for baseline RF and other covariates. The accelerated increase of sTNF α -R1 was strongly associated with the accelerated decline of eGFR ($\hat{\beta} = 0.25$, $p<0.001$). A similar pattern was observed for the association between the rate of increase of sTNF α -R2, IL-6, and HS-CRP; and the rate of eGFR change ($\hat{\beta} = -0.18$, $p<0.001$; $\hat{\beta} = -0.06$, $p=0.01$; $\hat{\beta} = -0.07$, $p=0.002$, respectively) in separate models (Table S5).

After adjustment for baseline eGFR and other covariates, we simultaneously entered the significant biomarkers' slopes in the model. sTNF α -R1 and sTNF α -R2 slopes were highly correlated ($r=0.98$); therefore, we excluded sTNF α -R2. The rate of sTNF α -R1 change remained significantly associated with the rate of eGFR change, so that 1 SD increase of TNF α -R1 is associated with 0.25 SD faster decline in eGFR ($\hat{\beta}=-0.25$, $p<0.001$; Table 2). Having CHF and using angiotensin-converting enzyme inhibitor or angiotensin II receptor blockers were not significantly associated with the rate of eGFR change ($p>0.7$). When we performed similar analyses for non-hypertensive individuals, both baseline association and the association of rate of sTNF α -R1 change with rate of change of eGFR remained significant (baseline: $\hat{\beta}=-0.23$, $p<0.001$; rate of change: $\hat{\beta}=-0.15$, $p<0.001$). Similarly, in these sensitivity analyses, the baseline sTNF α -R2 levels and the rate of sTNF α -R2 change were significantly associated with the rate of eGFR change ($\hat{\beta}=-0.06$, $p=0.001$; $\hat{\beta}=-0.13$ $p<0.001$, respectively).

Inflammatory biomarkers and incident CKD

We assessed whether sTNF α -R1 at previous visits predicted incident CKD at 3-, 6-, and 9-year follow-ups. Participants with high levels of sTNF α -R1 at a given visit (baseline, 3-year, or 6-year) had a significantly higher risk of incident CKD at a subsequent visit. Additionally, relative risks (RR) for incident CKD per SD increment of baseline TNF α -R1 were: 3-year RR: 1.3 (95%CI: 1.1 to 1.5), 6-year RR: 1.5 (95%CI: 1.1 to 2.2), and 9-year follow-ups RR: 1.6, (95%CI: 1.1 to 2.3). Similarly, SD increments of TNF α -R1 measured at the 3-year and 6-year visits were significantly associated with incident CKD at later visits (Table 3). We found similar results for TNF α -R2 and incident CKD. No other inflammatory biomarker significantly predicted incident CKD (Table S6).

Discussion

Our study shows that in the group of nondiabetic community-dwelling older adults with initially normal RF, higher baseline sTNF α receptors are associated with accelerated RF decline, and a greater likelihood of incident CKD. This association remained strong over the 9-year follow-up and was independent of SBP and new-onset DM. We also showed that greater increases of these inflammatory biomarkers were associated with faster decline of RF, independent of baseline RF, SBP, new-onset DM, and CHF.

Our results are similar to other studies showing the predictive roles of TNF α receptors for incident CKD. In contrast, a study conducted in a Norwegian population showed CRP as the inflammatory biomarker most strongly associated with incident CKD.¹⁸ That study, however, was limited to a younger population (middle age) with shorter follow-up time. Additionally, the data were collected from only two follow-up visits, which might have resulted in regression toward mean error.¹⁸ These authors also suggested that the previously reported association of TNF α -R2 with incident CKD could be secondary to decline in renal excretion. Our findings, however, are not consistent with this interpretation, as we evaluated the association of the baseline TNF α receptors and the rate of RF decline, independent of baseline RF. Moreover, our results were confirmed in lag-time models where the incident CKD was estimated independent of baseline RF and based only on high inflammation states

in the previous visits. These analytical methods should minimize the interference of renal excretion of inflammatory biomarkers.

Of note, most longitudinal studies that reported associations of TNF α -R1 or TNF α -R2 with the rate of RF decline were performed in participants who were significantly older than the Norwegian participants.^{15–18} While CRP is a marker of general inflammatory status, sTNF α -R2 is a tissue-specific biomarker usually expressed in endothelial cells and leukocytes³² while its expression in other tissues is increased in disease states.³³

Some studies have demonstrated that TNF- α and/or its circulating receptors are associated with reduced RF, but these studies have focused mainly on diabetic patients. One such investigation demonstrated that sTNF α receptors strongly predict stage 3 CKD in individuals with type-1 DM without proteinuria.³⁴ There is also evidence that sTNF α receptors predict end-stage renal disease in individuals with type 2 DM.³⁵ Furthermore, previous studies have found an association between high levels of sTNF α -R1 and diabetic nephropathy.^{34, 36, 37} Our results suggest that, independent of other risk factors, micro-inflammation emerges at a very early stage of RF decline and should be considered as a potential independent risk factor in older adults.^{15–17, 38} Among the inflammatory biomarkers tested, we found that sTNF α -R1 and sTNF α -R2 were the strongest and most consistent predictors of CKD. Reports indicate that TNF α induces cleavage of the TNF α receptors from the membrane receptor.³⁹ TNF α -R1 activates pathways downstream to NF- κ B, such as kinases of mitogen-activated protein kinase, or “death domain molecules,” resulting in IL-6 synthesis or apoptosis.^{40–42} sTNF α -R1 is considered an indicator of long-term activation of TNF α .³⁴ It has been shown that sTNF α -R1 also activates the apoptosis pathway through transforming growth factor beta-1.⁴³ Allugor et al. have reported that the R92Q mutation (rs4149584) increases the affinity of TNF to its receptor. In our study, all individuals were normal homozygotes for this variant.²³

Consistent with previous research, we found that increased levels of HS-CRP were associated with the prospective, accelerated decline of RF and CKD development, and increased CRP at the 6-year follow-up predicted incident CKD at the 9-year.¹⁸ These associations, however, were attenuated after adjustment for sTNF α -R1.

In our study, we found that sTNF α -R1 and sTNF α -R2 predict incident CKD. We used a categorical threshold of eGFR<60 ml/min/1.73m² as representing CKD, consistent with consensus definitions commonly utilized in large epidemiologic studies. We further adjusted for baseline eGFR to minimize bias from variation in initial RF.

Although IL-6 and TNF α have been frequently reported in association with age-related outcomes.^{44–45} The mechanisms that link circulating inflammatory biomarkers and CKD risk, however, remain hypothetical. sTNF α receptors have been shown to play a pivotal role in the development of tubulointerstitial fibrosis and, therefore, nephropathy.⁴⁶ Moreover, inflammatory-induced atherosclerosis or oxidative stress detected by these biomarkers might mediate RF decline, with the latter linked to increased sTNF α -R2.⁴⁵ Additionally, age-related chronic inflammation might have an important role in the development of glomerulosclerosis. In a study of kidney donors, Kremers et al. reported that older age was

associated with global glomerulosclerosis and higher odds of sclerosis in hypertensive donors.⁴⁷ Although these investigators did not measure inflammatory biomarkers, it is known that aging is associated with the development of inflammatory states. Mayr et al. demonstrated that angiotensin-II infusion in mice introduces a fibroinflammatory response that is followed by kidney tubulointerstitial collagen deposition mediated through the TNF-R1 signaling pathway and speculating that TNF α -R1 triggers collagen deposition.⁴⁸ Additionally, the significant association of lipocalin 2 in mesangial cells with sTNF α -R2 levels and impaired kidney function has been reported.³⁸ Therefore, a similar persistent age-related chronic inflammation might result in structural modification of glomerular and interstitial components of the kidneys, with resulting decline in RF.

Strengths and Limitations

Strengths of our study include the longitudinal design, with multiple repeated measures of RF and inflammatory biomarkers over a 9-year follow-up, and use of inverse probability weighting in the analysis to account for random missing values due to missing visits and truncation by death. Furthermore, due to the uncertainty of eGFR thresholds in aging, which identify true disease from age-related decline, we performed multiple analyses using both continuous measures of RF and an epidemiological-based threshold definition for CKD. These analyses demonstrate that the link between sTNF α receptors and RF is robust and independent of baseline RF. Lack of proteinuria measures in follow-up visits is a limitation. Consensus guidelines, however, define eGFR<60ml/min/1.73m² for more than 3 months as defining stage 3 or greater CKD, irrespective of proteinuria. In addition, albuminuria with preserved RF in older adults is primarily a manifestation of diabetic or hypertensive renal disease. Excluding the individuals with these morbidities in our study, the associations between TNF α receptors and the time-dependent slope and other RF outcomes remained significant.²⁶ One limitation of our study is that the follow-up intervals are 3 years apart. We operationalized time as a continuous variable to overcome this limitation for the annual slope of eGFR; incident CKD, though, is probably underestimated.

Conclusion

sTNF α receptors were the inflammatory biomarkers with the strongest association with the age-related decline of RF both at baseline and over time. Temporal changes in sTNF α receptors were associated with the rate of RF change. Moreover, these biomarkers predicted incident CKD independent of the main conventional risk factors and baseline RF. In contrast, in multivariate analyses, other inflammatory biomarkers, including IL-6, IL-18, and IL-1 β , were not consistently significantly associated with RF outcomes. These results suggest that higher levels of sTNF α receptors, in the absence of other chronic conditions such as DM, might be an early sign of impending, accelerated RF decline. Furthermore, these biomarkers could be used to identify individuals at elevated risk of CKD who could be candidates for interventions aimed at preventing progression of RF decline.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Effect size (standardized beta) from separate regression models examining associations of each log-transformed baseline inflammatory biomarker and longitudinal log-transformed renal function (eGFR), adjusted for covariates. sTNF α -R1 & 2: soluble tumor necrosis factor alpha-receptor 1 and 2, IL-6 (Interleukin-6), IL-1 β : Interleukin-1 beta, IL-1RA: Interleukin-1 receptor antagonist, IL-18: Interleukin-18, CRP: C-reactive protein.

Table 1

Baseline characteristics of total study participants, those with incident CKD (eGFR <60 anytime during the follow-up) vs. no-CKD (eGFR ≥ 60 ml/min/1.73 m²).

Baseline	Total n = 687	No CKD 60, n = 434 ^a	Incident CKD < 60, n = 188	p-Value
Demographic variables				
Age, mean yr ± SD	72.9 ± 7.1	71.4 ± 6.4	73.5 ± 6.2	0.001
Sex, %				
Male	46.0	45.6	43.6	0.64
Education, mean yr ± SD	5.6 ± 3.4	5.8 ± 3.2	5.6 ± 3.7	0.42
Marital status, %				
Not married	8.3	8.5	8.5	
Married	66.1	71.4	59.6	0.002
Widowed	25.6	20.1	31.9	
Smoking, %				
Never	58.1	58.1	59.0	0.77
Former	25.8	25.3	26.6	
Current	16.1	16.6	14.4	
NSAIDs, %				
Yes	41.9	41.4	43.3	0.65
Physical exam variables				
Systolic BP, mean ± SD	158.8 ± 19.2	157.5 ± 19.4	162.4 ± 18.5	0.003
Diastolic BP, mean ± SD	84.0 ± 8.4	83.6 ± 8.5	85.1 ± 8.2	0.04
ACEI, column %	19.21	18.3	19.6	0.60
ARBs, column %	1.86	1.8	2.39	0.50
CHF, column %	1.03	0.81	1.44	0.35
Base muscle mass area calf mm ² ± SD	71.1 ± 3.5	6286 ± 1252	6461 ± 1254	0.12
Laboratory				
Serum glucose, mg/dL	88.1 ± 10.7	88.1 ± 10.5	88.7 ± 10.9	0.50
Serum creatinine, mg/dL	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.001
Baseline eGFR ml/min/1.73m ²	76.4 ± 10.0	78.8 ± 9.8	71.8 ± 8.9	0.001
Annual eGFR slope ml/min/1.73 m ²	-0.32 ± 0.1	0.9 ± 0.1	-7.9 ± 0.3	0.001
Inflammatory biomarkers Median (IQR)	-0.50 ± 0.6	-0.2 ± 0.06	-2.1 ± 0.2	<0.001
sTNFα-R1 pg/mL	1253 (1037–1526)	1174 (977–1432)	1402 (1135–1571)	0.001
sTNFα-R2 pg/mL	2499 (2205–2914)	2377 (2091–2757)	2587 (2297–2961)	0.001
IL-1RA pg/mL	130.8 (95.4–176.3)	127.0 (94.0–169.0)	134.3 (94.2–180.4)	0.27
IL-1β pg/mL	0.12 (0.08–0.20)	0.12 (0.08–0.18)	0.13 (0.09–0.2)	0.10
IL-6 pg/mL	2.7 (1.9–3.7)	2.5 (1.9–3.5)	2.8 (1.9–3.7)	0.03
IL-18 pg/mL	367.7 (283.6–457.2)	360.4 (280.3–450.7)	65.8 (284.3–440.0)	0.73
HS-CRP mg/dL	2.5 (1.3–5.1)	2.2 (1.1–4.6)	2.8 (1.4–5.3)	0.03

Estimated glomerular filtration rate based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation

^aOf 687, 42 individuals died at 3-year follow-up and 23 missed 3-year follow-up and died at 6-year follow-up, therefore, not possible to quantify incident CKD for.

^b*p*-value based on non-parametric test: Kolmogorov-Smirnov or chi-square tests

NSAIDs: Nonsteroidal anti-inflammatory drugs

BP: blood pressure

ACEI: Angiotensin converting enzyme inhibitor

ARBs: Angiotensin II receptor blockers

CHF: Congestive heart failure

sTNF α -R1: soluble tumor necrosis factor alpha-1 receptor

sTNF α -R2: soluble tumor necrosis factor alpha-2 receptor

IL: Interleukin

HS-CRP: High sensitivity C-reactive protein

IL-1RA: Interleukin-1 receptor antagonist,

HS-CRP: high sensitive C-reactive protein

Column % is reported

IQR: Inter Quartile Range

Table 2

Association between the rates of all significant inflammatory biomarkers simultaneously entered in the model (slope of change of biomarkers over time) and the rate of renal function (slope of eGFR over time).

	Slope of eGFR over 9 years ^a	
	$\hat{\beta}$	<i>p</i>
TNF α -R1	-0.25(0.03)	<0.001
IL-6	-0.03(0.03)	0.50
HS-CRP	-0.01(0.02)	0.49
DM	-0.08(0.05)	0.57
SBP	-0.05(0.054)	0.02

^aSlope of eGFR: slope of estimated rate of glomerular filtration, the model is adjusted for baseline eGFR, age, sex, marital status, education, site of study, cancer, NSAIDs use, calf muscle area, SBP, new-onset DM, baseline systolic blood pressure (SBP); slopes of eGFR and all inflammatory biomarkers, calf muscle area and SBP are standardized; $\hat{\beta}$: standardized beta regression coefficient, presented per SD unit increase; SD: standard deviation

Table 3

Association of TNF α -R1 at each visit with incident CKD at subsequent Follow-up visits.

Time of TNF α -R1 Measurement	Year 3 Incident CKD RR (95% C	Year 6 Incident CKD RR (95% CI)	Year 9 Incident CKD RR (95% CI)
Baseline	1.3(1.1–1.5)	1.5(1.1–2.2)	1.6(1.1–2.3)
Year 3	-	2.0(1.5–2.6)	1.5(1.1–2.2)
Year 6	-	-	1.3(1.07–1.7)

Standardized TNF α -R1, RR: relative risk, CKD is defined as new development of eGFR <60 ml/min/1.73 m² at subsequent follow-up visit, adjusted for sex, marital status, education, site of study, and time relevant age, cancer, NSAID use, calf muscle area, SBP, new-onset DM