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Biomarkers of Immune Activation and Incident Kidney Failure With Replacement Therapy: Findings From the African American Study of Kidney Disease and Hypertension

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Abstract

Rationale & Objective: Immune activation is fundamental to the pathogenesis of many kidney diseases. Innate immune molecules such as soluble urokinase-type plasminogen activator receptor (suPAR) have been linked to incidence and progression of chronic kidney disease (CKD). Whether

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other biomarkers of immune activation are associated with incident kidney failure with replacement therapy (KFRT) in African Americans with non-diabetic kidney disease is unclear.

Study Design: Prospective cohort

Setting & Participants: African American Study of Kidney Disease and Hypertension participants with available baseline serum samples for biomarker measurement.

Predictors: Baseline serum soluble tumor necrosis factor receptor 1 and 2 (sTNFR1; sTNFR2), tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ) levels.

Outcomes: Incident KFRT, all-cause mortality.

Analytic Approach: Cox proportional hazards models.

Results: Among 500 participants with available samples, mean glomerular filtration rate was 44.7 ml/min/1.73 m^2 and median urine protein-to-creatinine ratio was 0.09 g/g at baseline. Over a median follow-up 9.6 years, there were 161 (32%) KFRT and 113 (23%) death events. In models adjusted for demographic and clinical factors and baseline kidney function, each two-fold higher baseline level of sTNFR1, sTNFR2, and TNF-α was associated with 3.66 (95% CI: 2.31,5.80), 2.29 (95% CI: 1.60,3.29), and 1.35-fold (95% CI: 1.07,1.71) greater risks of KFRT, respectively; in comparison, the association between suPAR and KFRT was 1.39 (95% CI: 1.04,1.86). These three biomarkers were also significantly associated with death (up to 2.2-fold higher risks per 2-fold higher baseline level; p≤0.01). IFN-γ was not associated with either outcome. None of the biomarkers modified the association of *APOL1* high-risk status (genetic risk factors for kidney disease among individuals of African ancestry) with KFRT (p-interaction>0.05).

Limitations: Limited generalizability to other ethnic groups or CKD etiologies.

Conclusions: Among African Americans with CKD attributed to hypertension, baseline levels of sTNFR1, sTNFR2, and TNF-α but not IFN-γ were associated with KFRT and mortality.

Keywords

sTNFR1; sTNFR2; TNF-α; IFN-γ; ESKD; biomarkers; *APOL1*; immune activation

INTRODUCTION

Chronic kidney disease (CKD) is an urgent public health problem that affects an estimated 697.5 million adults worldwide.[1] Progression of CKD to kidney failure with replacement therapy (KFRT) is associated with increased morbidity and mortality.[2,3] Understanding factors that promote kidney function decline is therefore paramount to improving the outcomes of patients with CKD.

Central to the pathogenesis of many types of kidney disease is activation of the innate and/or adaptive immune systems.[4–6] In the African American Study of Kidney Disease and Hypertension (AASK), higher baseline levels of soluble urokinase-type plasminogen activator receptor (suPAR), a biomarker of immune activation, were associated with increased risks of CKD progression and incident KFRT.[7] Biomarkers of the tumor necrosis factor signaling pathway also appear promising in identifying individuals at risk of

progressive CKD.[8–11] Among patients with type 1 diabetes, higher baseline plasma levels of soluble tumor necrosis factor receptors 1 and 2 (sTNFR1, sTNFR2) were associated with 2.5 to 3.0-fold higher risks of incident CKD Stage G3+ (comparing fourth vs. first through third quartiles)[8] whereas in patients with type 2 diabetes, each quartile increase in baseline plasma sTNFR1 and sTNFR2 was associated with 9.8 and 6.0-fold higher risks of developing KFRT, respectively.[9] In two large trials of patients with type 2 diabetes, baseline plasma levels of sTNFR1 and sTNFR2 significantly improved prediction of kidney function decline beyond traditional clinical factors.[10] In the Chronic Renal Insufficiency Cohort (CRIC; 48% with diabetes), the highest quartile of baseline plasma tumor necrosis factor alpha (TNF-α) was associated with a 42% higher risk of CKD progression, defined as a ≥50% decline in estimated glomerular filtration rate (eGFR) or KFRT, compared to the first quartile.[11] Although substantial, the associations of sTNFR1, sTNFR2, and TNF-α with CKD have primarily been reported in individuals with diabetes and of European descent.[8–11] Whether these associations also exist in non-diabetic kidney disease and/or African Americans is unclear and warrants further investigation.

Immune activation may also have an important role in *APOL1*-associated kidney disease. Risk variants in the *APOL1* gene (G1 and G2), are found almost exclusively in individuals of African ancestry and have emerged as risk factors for various kidney diseases (e.g., HIV-associated nephropathy, collapsing lupus glomerulopathy) and CKD progression.[12–18] However, not all individuals with the *APOL1* high-risk genotypes develop kidney disease, thus suggesting that a “second hit” is necessary.[15,18,19] To date, *in vitro* and animal model studies have implicated that this “second hit” involves the activation of inflammatory pathways.[4,20,21] *In vivo*, interferon gamma (IFN-γ) and TNF-α increase APOL1 expression in endothelial cells and podocytes.[20–22] In mouse models, increased APOL1 expression, particularly the G1 and G2 risk variants, is associated with azotemia and albuminuria.[21] To our knowledge, there have been no studies in humans examining whether IFN-γ and TNF-α modify the association of *APOL1* with progressive kidney disease.

Utilizing data and stored serum samples from AASK, a cohort of African Americans with hypertension-attributed CKD, we measured baseline levels of sTNFR1, sTNFR2, TNF-α, and IFN-γ. We hypothesized that higher concentrations of each biomarker would be associated with greater risks of KFRT, CKD progression, and all-cause mortality. We further hypothesized that these biomarkers would augment *APOL1*-associated risks for KFRT and CKD progression.

METHODS

Study Population

We included 500 AASK trial participants with available baseline serum samples. Details regarding AASK have previously been described.[23–25] Briefly, AASK was a 3×2 factorial, double-blinded, randomized, controlled trial wherein 1,094 African Americans with hypertension-attributed CKD were randomized to one of three blood pressure (BP) medications (ramipril, metoprolol, or amlodipine) and one of two BP goals (mean arterial pressure of 102–107 mmHg or ≤92 mmHg). Inclusion criteria included ages 18–70 years, diastolic BP >95 mmHg, and ^{125}I-iothalamate GFR 20–65 ml/min/1.73 m^2. Exclusion

criteria included diabetes, urine protein-to-creatinine ratio (PCR) >2.5 g/g, or CKD etiology other than hypertension.[23–25] Trial participants were enrolled from February 1995 to September 1998 and followed until September 2001, the pre-specified end date of the trial.[23,24] Participants without KFRT were then invited to the cohort phase of AASK, which spanned from April 2002 to June 2007. During this second phase, all participants received ramipril with a BP goal of <140/90 mmHg, and after 2004, <130/80 mmHg due to changes in national guidelines.[24] Informed consent was obtained and protocols were approved by institutional review boards at each participating site.[23,24]

Biomarker Measurements

We measured biomarkers of immune activation from stored serum samples collected at the AASK trial baseline visit. Measurements were performed from December 2019 to January 2020 using Meso Scale Discovery assays (Meso Scale Diagnostics; Rockville, Maryland), which combines electrochemiluminescence with multiarray technology, by personnel blinded to participant data. sTNFR1 and sTNFR2 were measured in a 2-plex plate; TNF-α and IFN-γ were measured together on a separate plate. Inter-assay coefficients of variation, determined from 6% duplicate samples, were: sTNFR1=3.33%, sTNFR2=2.96%, TNF-α=7.52%, and IFN-γ=6.17%. Baseline serum suPAR levels were measured in 2017 using the suPARnostic® ELISA kit (Virogates; Copenhagen, Denmark).[7]

Genotyping of *APOL1* Risk Variants and Ancestry Informative Markers

A subset of participants were genotyped for the *APOL1* risk variants and 140 ancestry informative markers in an ancillary study.[15] Genotyping for G1 (rs73885319 and rs60910145) and G2 (rs71785313) were performed using ABI Taqman (Applied Biosystems; Foster City, California). *APOL1* high-risk status was defined as 2 copies of the risk variants (i.e., G1/G1, G2/G2, or G1/G2) and low-risk status as 1 or no copies (i.e., G1/G0, G2/G0, or G0/G0).[12–15,19] Percentage of European ancestry was estimated via ANCESTRYMAP.[15]

Outcomes

The primary outcome was incident KFRT, defined as initiation of chronic dialysis or kidney transplantation. Secondary outcomes included all-cause mortality and CKD progression, defined as a doubling of serum creatinine or KFRT. Serum creatinine was measured at a central laboratory at 6-month intervals during both phases of AASK.[23,24]

Covariates

At the screening visit (prior to randomization), each participant underwent 3 baseline BP measurements in a seated position after >5 minutes of rest using a Hawksley random zero sphygmomanometer. The latter two measurements were then averaged.[23] GFR was ascertained via direct measurement of renal ^{125}I-iothalamate clearance.[23] Urine protein and urine creatinine were measured at a central laboratory using the modified Jaffe reaction and pyrogallol red technique, respectively, from 24-hour urine specimens and the ratio taken to determine urine PCR.[24] Serum high-sensitivity c-reactive protein (hsCRP) was measured at a central laboratory by nephelometry (Dade Behring BN1).[26]

Statistical Analysis

Baseline characteristics by biomarker tertiles and *APOL1* risk status were compared using ANOVA, Wilcoxon rank-sum, or Kruskal-Wallis tests for continuous variables and Pearson's chi-squared test for categorical variables. Distributions of continuous variables were assessed and if skewed, $\log_2$-transformed to achieve more normal distributions (e.g., sTNFR1, sTNFR2, TNF-α, IFN-γ, suPAR, urine PCR, hs-CRP). To evaluate the association of each biomarker with KFRT, Cox models were constructed. Model 0 was unadjusted. Model 1 adjusted for demographic factors (age, sex). Model 2 further adjusted for clinical factors (baseline systolic BP, body mass index, current smoking). Model 3 further adjusted for baseline GFR. Model 4, our primary model, further adjusted for baseline $\log_2$-transformed urine PCR. Among individuals with *APOL1* genotyping, Model 5 further adjusted for *APOL1* risk status and European ancestry. Each analysis was repeated for the outcomes of all-cause mortality and CKD progression. In sensitivity analyses, we accounted for randomization groups and hsCRP, a general marker of inflammation. We also performed competing risks analyses, based on the method of Fine and Gray, treating death as a competing risk.[27] For comparison purposes, we evaluated associations between suPAR and KFRT in our study population, using $\log_2$-transformed suPAR as the exposure. Participants were censored at death or on June 30, 2007.

We included interaction terms between each biomarker (as a $\log_2$-transformed variable) and categories of *APOL1* risk status, age, sex, systolic BP, GFR, and urine PCR to evaluate for effect modification. To assess the predictive value of adding $\log_2$-transformed biomarkers to a clinical model, we calculated the Harrell's C-statistic for the following models: 1) Clinical (Model 4); 2) Clinical+sTNFR1; 3) Clinical+sTNFR1+TNF-α; 4) Clinical +sTNFR1+suPAR; and 5) Clinical+sTNFR1+TNF-α+suPAR. Differences in the Harrell's C statistic for each model with the biomarker(s) versus the clinical model were then determined. Analyses were conducted with Stata 15.1 software (StataCorp LLC; College Station, Texas).

RESULTS

Baseline Characteristics

Among 1,094 AASK trial participants, 500 had baseline serum samples available for biomarker measurement and comprised our study population (Figure 1). Participants without available samples had slightly higher mean GFR (46.4 vs. 44.7 ml/min/1.73 m^2) and suPAR levels (4,487 vs. 4,417 pg/mL) compared to those included in our study. Otherwise, the two groups were alike (Supplementary Table 1).

At baseline, the mean age was 54.1 ± 10.6 years, 37% were female, mean GFR was 44.7 ± 12.7 ml/min/1.73 m^2, and median urine PCR was 0.09 (Interquartile range [IQR] 0.03 to 0.39) g/g. Median (IQR) levels of each biomarker were as follows: sTNFR1: 2,875 (2,170 to 3,905) pg/mL, sTNFR2: 13,021 (9,345 to 17,155) pg/mL, TNF-α: 2.92 (2.20 to 3.95) pg/mL, and IFN-γ: 5.51 (3.81 to 8.69) pg/mL. Participants in the highest sTNFR1 tertile were significantly younger and had worse kidney function measures (i.e., GFR, urine PCR) compared to participants in lower tertiles (Table 1). Higher tertiles of sTNFR1 also had

higher median levels of other biomarkers of immune activation, including sTNFR2, TNF-α, and suPAR. Participant characteristics by tertiles of the other biomarkers are presented in Supplementary Tables 2–4. sTNFR1 and sTNFR2 were highly correlated with each other (correlation of 0.88) and moderately correlated with TNF-α and suPAR (correlations of 0.42 to 0.55; Table 2).

Among participants with genotyping, 87 (26%) had *APOL1* high-risk status and 246 (74%) had low-risk status. Participants with *APOL1* high-risk status were younger, had lower mean systolic BP and GFR, and had higher median urine PCR, serum sTNFR1, sTNFR2, and suPAR compared to participants with low-risk status. Otherwise, the two *APOL1* risk groups were similar at baseline, including with respect to TNF-α and IFN-γ levels (Supplementary Table 5).

Associations of Biomarkers with KFRT and CKD Progression

Over a median follow-up of 8.5 years, 161 participants developed KFRT. In unadjusted analyses, each 2-fold higher baseline level of sTNFR1 was associated with an 8.10-fold greater risk of incident KFRT (95% CI: 6.15, 10.66). This association was robust to adjustment for demographic and clinical factors, and attenuated but remained statistically significant upon further adjustment for baseline GFR and proteinuria (HR=3.66; 95% CI: 2.31, 5.80). Similarly, each 2-fold higher baseline level of sTNFR2 was associated with 5.09 (95% CI: 4.03, 6.43) and 2.29-fold (95% CI: 1.60, 3.29) greater risks of incident KFRT in unadjusted model and Model 4, respectively. The association of TNF-α with incident KFRT was smaller though still significant (HR=1.88 [95% CI: 1.54, 2.29] for unadjusted model; HR=1.35 [95% CI: 1.07, 1.71] for Model 4) whereas IFN-γ was not associated with incident KFRT in any of the models (all $p>0.05$; Table 3). In comparison, the association between suPAR and KFRT in this population was HR=1.39 (95% CI: 1.04, 1.86) per two-fold higher baseline level. Conclusions did not change upon further adjustment for randomized treatment groups or hs-CRP (Supplementary Table 6). In Kaplan-Meier curves, higher tertiles of baseline sTNFR1, sTNFR2, and TNF-α were associated with higher risk of incident KFRT (Figure 2). In additional analyses, the association of sTNFR2 with KFRT appeared stronger among older participants (p-interaction=0.008) and those without baseline proteinuria (p-interaction=0.045). Otherwise, associations did not differ significantly by subgroups (Supplementary Table 7). Finally, accounting for the competing risk of death yielded similar results with the exception of TNF-α, for which the subdistribution HR lost statistical significance but direction of association remained the same (Supplementary Table 8).

When considering the secondary outcome of CKD progression, there were 196 events over a median follow-up 7.3 years. In unadjusted models, each 2-fold higher baseline level of sTNFR1, sTNFR2, and TNF-α was associated with 6.10 (95% CI: 4.76, 7.82), 4.00 (95% CI: 3.22, 4.95), and 1.76-fold (95% CI: 1.47, 2.11) greater risks for CKD progression, respectively. After adjusting for demographic/clinical factors and baseline kidney function, these associations were attenuated but remained significant (sTNFR1: HR=2.96 [95% CI 1.93, 4.55]; sTNFR2: HR=1.85 [95% CI: 1.32, 2.58]; TNF-α: HR=1.31 [95% CI: 1.06,

1.62] per 2-fold higher baseline level). As in our primary analyses, there was no association between IFN-γ and CKD progression (Supplementary Table 6 and 9).

Associations of Biomarkers with All-cause Mortality

There were 113 deaths over a median follow-up of 9.6 years. In unadjusted models, each 2-fold higher baseline level of sTNFR1, sTNFR2, and TNF-α was associated with 1.7 to 1.8-fold greater risks of all-cause mortality (all p≤0.002). After accounting for demographic/clinical factors and baseline kidney function, these associations strengthened, with each 2-fold higher baseline level of sTNFR1, sTNFR2, TNF-α being associated with 2.0 to 2.2-fold higher risks of death (all p≤0.01). Baseline IFN-γ was not associated with all-cause mortality (Table 4; Supplementary Table 6).

APOL1 Risk Genotypes and Immune Activation

Among participants with available genotyping (n=333), each 2-fold higher baseline level of sTNFR1 was associated with a significantly increased risk of KFRT (HR=3.83; 95% CI: 2.21, 6.61), CKD progression (HR=2.76; 95% CI: 1.68, 4.54), and mortality (HR=2.88; 95% CI: 1.31, 6.35), after adjusting for *APOL1* risk status and European ancestry. Similar trends were observed for sTNFR2 and TNF-α but not IFN-γ (Tables 3–4; Supplementary Table 9). There was no evidence of interaction between *APOL1* high-risk status and sTNFR1, sTNFR2, TNF-α, or IFN-γ for the outcomes of KFRT (p-interaction=0.51, 0.53, 0.98, and 0.43, respectively) and CKD progression (p-interaction=0.86, 0.92, 0.75, and 0.38, respectively).

Improvement in Prediction Model Discrimination by Biomarkers

The C-statistic for the fully adjusted clinical model in predicting KFRT was excellent at 0.849 (95% CI: 0.820, 0.878). Adding sTNFR1, the biomarker with the strongest association, to the clinical model led to a small but statistically significant improvement in the C-statistic at 0.860 (95% CI: 0.833, 0.887; difference of 0.011; 95% CI: 0.001, 0.021). Addition of TNF-α, suPAR, or both to the model did not further improve discrimination measures (C-statistics of 0.860, 0.860, and 0.860, respectively; Table 5).

DISCUSSION

In this study of African Americans with hypertension-attributed CKD, higher baseline levels of sTNFR1, sTNFR2, TNF-α, but not IFN-γ were independently associated with increased risks of KFRT, CKD progression, and all-cause mortality. None of the biomarkers that we examined, however, modified the association of *APOL1* high-risk status with KFRT or CKD progression. We also report that the addition of sTNFR1, the biomarker with the strongest associations in our study population, to a clinical model improved KFRT risk prediction, albeit by a small magnitude. Further inclusion of additional biomarkers did not. Taken together, our findings support the hypothesis that the tumor necrosis factor signaling pathway plays an important role in CKD progression in African Americans with non-diabetic kidney disease.

To date, few studies have investigated the clinical significance of sTNFR1 and sTNFR2 in non-diabetic kidney disease, with little representation of African Americans. In the Cholesterol and Recurrent Events trial (14% with diabetes; 2% African American), higher sTNFR1 were associated with faster eGFR decline (−0.49 ml/min/1.73 m^2 per year comparing highest vs. lowest tertiles).[28] Similarly, in the Multi-Ethnic Study of Atherosclerosis (27% with impaired fasting glucose or diabetes; 24% African American), each standard deviation higher sTNFR1 was associated with a 43% higher risk of 40% decline in eGFR.[29] In the Beaver Dam CKD study (9% with diabetes; 98% Caucasian), the highest tertile of sTNFR2 was associated with a 2.1-fold higher risk of incident CKD compared to the lowest tertile.[30] In contrast, Schei *et al.* reported in a general population cohort of Norwegians without CKD (0% diabetes) that higher baseline levels of sTNFR2 were associated with slower declines in GFR (+0.09 ml/min/1.73 m^2/year per standard deviation increase).[31] More recently, in the CKD in Children cohort (median age 11 years; 20% African American), the highest quartiles of sTNFR1 and sTNFR2 were associated with 4.1-fold and 2.6-fold greater risks of CKD progression, defined as a 50% decline in eGFR or KFRT, compared to the lowest quartiles.[32] The present study adds to this literature by demonstrating strong associations of sTNFR1, sTNFR2, and TNF-α with risks of KFRT, CKD progression, and mortality among African Americans with non-diabetic kidney disease.

With a 2 to 4-fold higher lifetime risk of CKD G4+ and KFRT compared to Caucasians, African Americans carry an excess burden of progressive kidney disease.[33] The *APOL1* high-risk genotypes, present in ~13% of African Americans, account for some of the racial disparities in advanced CKD.[12,13,34] Parsa *et al.* reported that AASK participants with the *APOL1* high-risk status were 2.16 and 1.88-fold more likely to develop KFRT and CKD progression, respectively, compared to their counterparts with the low-risk status.[15] Still, 42% of individuals with the *APOL1* high-risk status did not experience CKD progression over a median follow-up of 9 years.[15] We previously described potential interactive effects of suPAR with *APOL1* risk status, where *APOL1* high-risk status was associated with faster eGFR decline when suPAR was >3,000 pg/mL but not <3,000 pg/mL in AASK and the Emory Cardiovascular Biobank.[4] However, suPAR did not modify the association of *APOL1* high-risk status with KFRT or CKD progression in AASK alone.[7] We expand upon these findings by reporting that higher baseline levels of other biomarkers of immune activation (i.e., sTNFR1, sTNFR2, TNF-α, IFN-γ) also did not modify the association of *APOL1* high-risk status with KFRT or CKD progression, and that sTNFR1 and sTNFR2 in particular were moderately correlated with suPAR.

To our knowledge, only one other study has examined the association of sTNFR1 and sTNFR2 with kidney outcomes in the context of *APOL1*. Utilizing data from Bio*Me* (16% with diabetes), an electronic-medical record-based retrospective cohort, Nadkarni *et al.* reported that each 2-fold higher baseline level of sTNFR1 and sTNFR2 was independently associated with a 2.0 and 1.5-fold higher risk of a composite renal outcome comprising of a sustained decline in eGFR by ≥40% or KFRT.[35] Their study was limited to African Americans with the *APOL1* high-risk status, and thus did not provide insight on whether sTNFR1 or sTNFR2 modify the association of *APOL1* high-risk status with CKD progression.[35] The results of our study, which included both individuals with the *APOL1*

high- and low-risk genotypes, suggest that the associations of sTNFR1 and sTNFR2 with progressive kidney disease may not differ by *APOL1* risk status.

We hypothesized that higher baseline levels of IFN-γ would augment the *APOL1*-associated risk for worsening CKD because prior *in vitro* studies showed that this inflammatory cytokine increases *APOL1* expression in endothelial cells and podocytes, both cell types found in the human kidney.[20,21] Moreover, expression of the *APOL1* G1 and G2 variants increases cytotoxicity in a dose-dependent manner.[21] In patients with HIV-associated nephropathy and lupus collapsing glomerulopathy, two other entities known to be associated with *APOL1* high-risk status, tubuloreticular inclusions are often seen on kidney biopsies.[36–39] These electron-dense deposits are considered to be interferon footprints, likely reflecting high interferon states.[36,37] Nichols *et al.* also described a series of 7 patients who developed focal segmental glomerulosclerosis after receiving interferon therapy and were all found to have 2 *APOL1* risk alleles.[20] Despite this prior evidence that IFN-γ may be a "second hit," we found no association between baseline IFN-γ levels and KFRT, CKD progression, or all-cause mortality and no interactive effects of IFN-γ with *APOL1* high-risk status for any of these outcomes. Perhaps, biomarkers measured at baseline may not be the biologically relevant time period to study with regards to *APOL1*-mediated kidney disease.

Our findings have potential implications. In the research arena, sTNFR1, sTNFR2, and/or TNF-α could be used to enrich recruitment of patients with CKD to clinical trials. Identifying individuals who are more likely to experience the outcome of interest (e.g., KFRT, CKD progression, or mortality) could reduce the number of participants needed or shorten the duration of the trial. Clinically, patients with higher levels of these biomarkers may benefit from intensification of conventional CKD treatments.

Strengths of our study include the prospective design of the AASK trial and cohort, long duration of follow-up (up to 12.2 years), direct measurement of GFR, consideration of multiple biomarkers, and evaluation of the interactive effects of immune activation with *APOL1* risk status. Our study also has limitations. First, given that AASK comprised African Americans with hypertension-attributed CKD, our results may not be generalizable to other ethnic groups or CKD etiologies. Although AASK excluded individuals with baseline diabetes or urine PCR >2.5 g/g, prior studies have shown strong associations in these other populations.[8–11,29] Second, our sample size was relatively small, especially for our analyses involving *APOL1*. We may have been underpowered to detect an interaction between our biomarkers of immune activation and *APOL1* risk status. Third, a "second hit" occurring early in the disease process would not be captured in our study population because moderate to severe CKD was already present at the time of enrollment. Fourth, although the results suggest a strong association between biomarkers and adverse outcomes, they do not imply causality. An intervention which lowers these biomarkers would not necessarily be expected to improve CKD prognosis.

In conclusion, among African Americans with CKD attributed to hypertension, baseline serum levels of sTNFR1, sTFNR2, and TNF-α were associated with adverse kidney outcomes and mortality, with sTNFR1 appearing to have the strongest associations. Future

studies are needed to determine the clinical utility of measuring and/or targeting these biomarkers in both patient care and clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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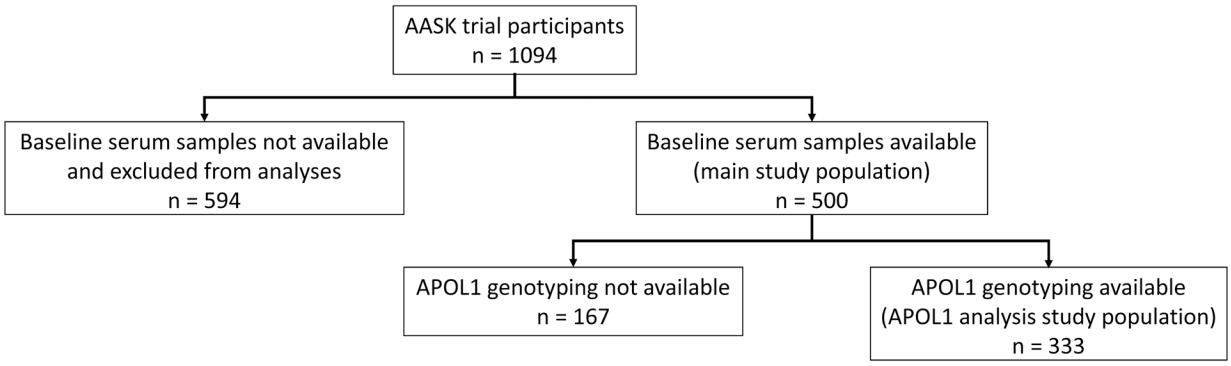

Figure 1:
Flowchart of study population included in current study.

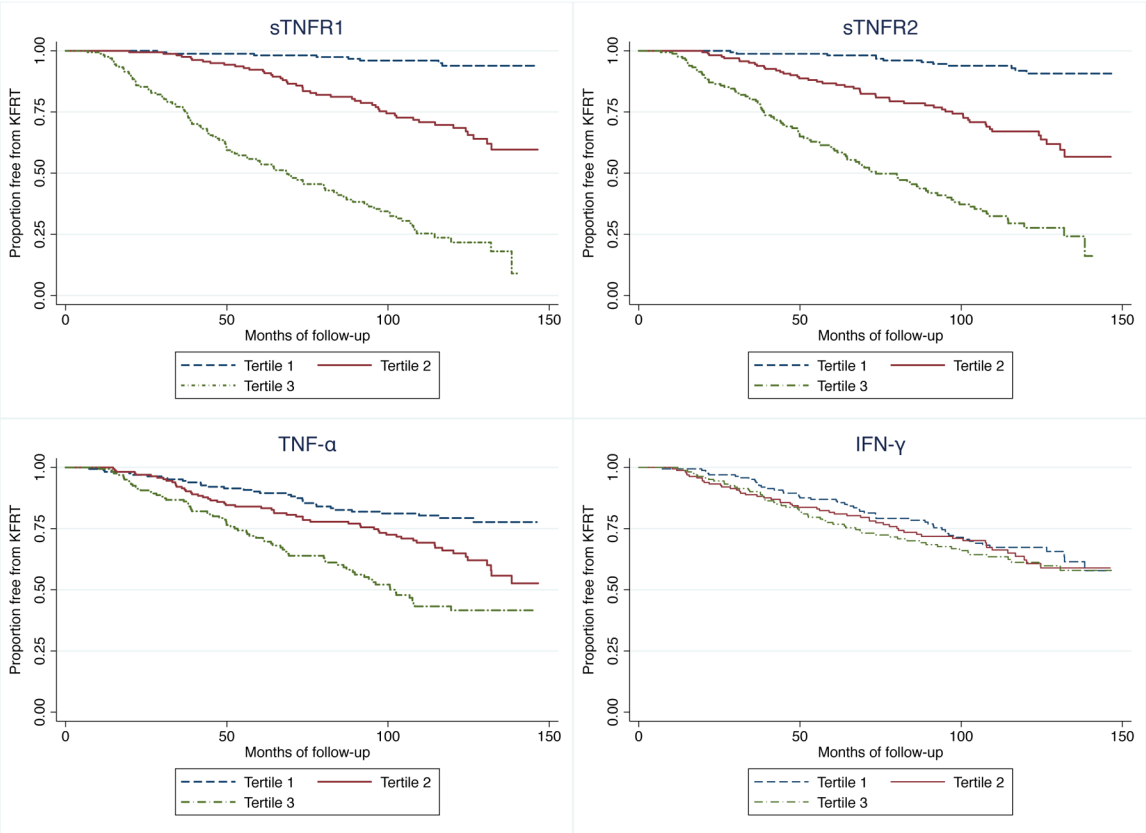

Figure 2:

Kaplan-Meier survival estimates of kidney failure with replacement therapy, by biomarker tertiles.

Table 1:

Baseline characteristics by sTNFR1 tertiles.

Characteristic	Tertile 1 n=167	Tertile 2 n=167	Tertile 3 n=166	p-value
Range, pg/mL	1,267 to 2,372	2,374 to 3,428	3,445 to 9,168	
Age, years	58.1 ± 9.0	53.1 ± 10.6	51.0 ± 10.9	<0.001
Female	67 (40%)	64 (38%)	56 (34%)	0.46
APOL1 risk status				
Low	92 (82%)	83 (71%)	71 (68%)	0.05
High	20 (18%)	34 (29%)	33 (32%)	
% European ancestry	17.9 ± 15.2	14.6 ± 10.6	17.8 ± 11.8	0.09
Years of hypertension	14.4 ± 9.8	14.5 ± 9.5	13.2 ± 10.5	0.40
History of heart disease	87 (52%)	90 (54%)	75 (45%)	0.24
Systolic BP, mm Hg	149 ± 25	152 ± 25	153 ± 23	0.39
BMI, kg/m^2	30.1 ± 5.7	31.5 ± 6.4	31.0 ± 7.3	0.11
Current smoking	40 (24%)	44 (26%)	48 (29%)	0.59
GFR, ml/min/1.73 m^2	54.7 ± 7.7	47.7 ± 9.1	31.7 ± 8.0	<0.001
Urine PCR, g/g	0.03 (0.02 to 0.06)	0.10 (0.03 to 0.32)	0.37 (0.12 to 0.99)	<0.001
hsCRP, mg/dL	0.32 (0.16 to 0.71)	0.50 (0.24 to 0.97)	0.46 (0.18 to 0.91)	0.01
sTNFR2, pg/mL	8,651 (7,487 to 9,884)	13,042 (10,962 to 14,735)	18,972 (15,936 to 22,917)	<0.001
TNF-α, pg/mL	2.40 (1.78 to 2.92)	3.03 (2.27 to 3.93)	3.74 (2.67 to 4.95)	<0.001
IFN-γ, pg/mL	5.23 (3.72 to 7.55)	5.55 (3.73 to 9.01)	5.73 (3.95 to 11.68)	0.12
suPAR, pg/mL	3,468 (2,821 to 4,334)	4,463 (3,352 to 5,583)	5,561 (4,618 to 7,116)	<0.001
Log_2(sTNFR2)	13.07 ± 0.32	13.65 ± 0.32	14.24 ± 0.39	<0.001
Log_2(TNF-α)	1.23 ± 0.61	1.56 ± 0.60	1.90 ± 0.67	<0.001
Log_2(IFN-γ)	2.47 ± 0.86	2.67 ± 1.15	2.86 ± 1.43	0.01
Log_2(suPAR)	11.69 ± 0.61	12.11 ± 0.57	12.47 ± 0.55	<0.001
BP goal				
Intensive	87 (52%)	79 (47%)	84 (51%)	0.67
Standard	80 (48%)	88 (53%)	82 (49%)	
Drug group				
Ramipril	69 (41%)	59 (35%)	67 (40%)	0.70
Metoprolol	69 (41%)	70 (42%)	67 (40%)	
Amlodipine	29 (17%)	38 (23%)	32 (19%)	

Data presented as mean ± standard deviation; number (percent); median (interquartile range).

Abbreviations: BP=blood pressure; BMI=body mass index; GFR=glomerular filtration rate; PCR=protein-to-creatinine ratio; hsCRP=high-sensitivity c-reactive protein; sTNFR1=soluble tumor necrosis factor receptor 1; sTNFR2=soluble tumor necrosis factor receptor 2; TNF-α=tumor necrosis factor alpha; IFN-γ=interferon gamma; suPAR=soluble urokinase-type plasminogen activator receptor.

Data missing for the following variables: *APOL1* (n=167); European ancestry (n=167); Years of hypertension (n=3); suPAR (n=14); $\log_2$(suPAR) (n=14)

Table 2:

Pearson correlations of $\log_2$-transformed biomarkers of immune activation.

	sTNFR1	sTNFR2	TNF-α	IFN-γ	suPAR
sTNFR1	1.00				
sTNFR2	0.88	1.00			
TNF-α	0.42	0.48	1.00		
IFN-γ	0.14	0.28	0.28	1.00	
suPAR	0.53	0.55	0.31	0.22	1.00

Abbreviations: sTNFR1=soluble tumor necrosis factor receptor 1; sTNFR2=soluble tumor necrosis factor receptor 2; TNF-α=tumor necrosis factor alpha; IFN-γ=interferon gamma; suPAR=soluble urokinase-type plasminogen activator receptor.

Table 3:

Associations of log_2-transformed biomarkers of immune activation with kidney failure with replacement therapy in AASK.

Model	n	events	sTNFR1		sTNFR2		TNF-α		IFN-γ	
			Hazard Ratio (95% CI)	p-value	Hazard Ratio (95% CI)	p-value	Hazard Ratio (95% CI)	p-value	Hazard Ratio (95% CI)	p-value
Model 0: Unadjusted	500	161	8.10 (6.15, 10.66)	<0.001	5.09 (4.03, 6.43)	<0.001	1.88 (1.54, 2.29)	<0.001	1.11 (0.98, 1.25)	0.10
Model 1: Adjusted for age and sex	500	161	7.44 (5.59, 9.89)	<0.001	4.63 (3.63, 5.91)	<0.001	1.69 (1.39, 2.07)	<0.001	1.08 (0.96, 1.22)	0.19
Model 2: Model 1 + systolic BP, BMI, and current smoking	500	161	8.30 (6.06, 11.35)	<0.001	4.98 (3.82, 6.50)	<0.001	1.69 (1.39, 2.07)	<0.001	1.08 (0.96, 1.22)	0.19
Model 3: Model 2 + GFR	500	161	4.90 (3.16, 7.62)	<0.001	2.76 (1.97, 3.86)	<0.001	1.31 (1.05, 1.64)	0.02	1.09 (0.96, 1.23)	0.18
Model 4: Model 3 + log_2(urine PCR)	500	161	3.66 (2.31, 5.80)	<0.001	2.29 (1.60, 3.29)	<0.001	1.35 (1.07, 1.71)	0.01	1.03 (0.91, 1.16)	0.69
Model 5: Model 4 + *APOL1* risk status and European ancestry	333	112	3.83 (2.21, 6.61)	<0.001	2.53 (1.63, 3.95)	<0.001	1.28 (0.97, 1.70)	0.09	1.02 (0.88, 1.18)	0.81

Hazard ratios are per 2-fold higher baseline level of each biomarker.

Abbreviations: BP=blood pressure; GFR=glomerular filtration rate; PCR=protein-to-creatinine ratio; sTNFR1=soluble tumor necrosis factor receptor 1; sTNFR2=soluble tumor necrosis factor receptor 2; TNF-α=tumor necrosis factor alpha; IFN-γ=interferon gamma.

Table 4:

Associations of log_2-transformed biomarkers of immune activation with all-cause mortality in AASK.

Model	n	events	sTNFR1		sTNFR2		TNF-α		IFN-γ	
			Hazard Ratio (95% CI)	p-value	Hazard Ratio (95% CI)	p-value	Hazard Ratio (95% CI)	p-value	Hazard Ratio (95% CI)	p-value
Model 0: Unadjusted	500	113	1.65 (1.20, 2.27)	0.002	1.66 (1.23, 2.25)	0.001	1.83 (1.42, 2.34)	<0.001	1.02 (0.87, 1.19)	0.82
Model 1: Adjusted for age and sex	500	113	2.02 (1.46, 2.80)	<0.001	2.10 (1.54, 2.87)	<0.001	2.16 (1.64, 2.83)	<0.001	1.08 (0.92, 1.27)	0.33
Model 2: Model 1 + systolic BP, BMI, and current smoking	500	113	1.90 (1.38, 2.63)	<0.001	1.95 (1.44, 2.65)	<0.001	2.07 (1.57, 2.74)	<0.001	1.10 (0.94, 1.30)	0.23
Model 3: Model 2 + GFR	500	113	2.00 (1.18, 3.39)	0.01	1.98 (1.29, 3.03)	0.002	1.95 (1.44, 2.62)	<0.001	1.09 (0.93, 1.29)	0.29
Model 4: Model 3 + log_2(urine PCR)	500	113	2.21 (1.26, 3.85)	0.01	2.07 (1.34, 3.20)	0.001	1.95 (1.45, 2.62)	<0.001	1.09 (0.93, 1.29)	0.29
Model 5: Model 4 + *APOL1* risk status and European ancestry	333	55	2.88 (1.31, 6.35)	0.01	2.44 (1.31, 4.54)	0.01	2.14 (1.42, 3.23)	<0.001	1.23 (0.99, 1.52)	0.06

Hazard ratios are per 2-fold higher baseline level of each biomarker.

Abbreviations: BP=blood pressure; GFR=glomerular filtration rate; PCR= protein-to-creatinine ratio; sTNFR1=soluble tumor necrosis factor receptor 1; sTNFR2=soluble tumor necrosis factor receptor 2; TNF-α=tumor necrosis factor alpha; IFN-γ=interferon gamma.

Table 5:

Harrell's C statistic for clinical models ± biomarkers in predicting KFRT.

Model	Harrell's C Statistic (95% CI)	Difference in C Statistic (95% CI)
Clinical Model: adjusted for age, sex, systolic BP, BMI, current smoking, GFR, and $\log_2$(urine PCR)	0.849 (0.820, 0.878)	Ref
Clinical Model + $\log_2$(sTNFR1)	0.860 (0.833, 0.887)	0.011 (0.001, 0.021)
Clinical Model + $\log_2$(sTNFR1) + $\log_2$(TNF-α)	0.860 (0.834, 0.887)	0.011 (0.002, 0.021)
Clinical Model + $\log_2$(sTNFR1) + $\log_2$(suPAR)	0.860 (0.833, 0.887)	0.011 (0.001, 0.021)
Clinical Model + $\log_2$(sTNFR1) + $\log_2$(TNF-α) + $\log_2$(suPAR)	0.860 (0.833, 0.887)	0.011 (0.001, 0.021)

Abbreviations: BP=blood pressure; GFR=glomerular filtration rate; PCR=protein-to-creatinine ratio; sTNFR1=soluble tumor necrosis factor receptor 1; TNF-α=tumor necrosis factor alpha; IFN-γ=interferon gamma; suPAR=soluble urokinase-type plasminogen activator receptor.