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## Inflammation and Apparent Treatment Resistant Hypertension in Patients with Chronic Kidney Disease – The Results from the CRIC Study

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### Abstract

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Disclosure  
None

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Apparent treatment-resistant hypertension (ATRH) is highly prevalent and associated with cardiovascular disease (CVD) risk in patients with chronic kidney disease (CKD). We analyzed the association of inflammatory biomarkers with ATRH and its complications in CKD patients. ATRH was defined as blood pressure (BP)  $\geq 140/90$  mm Hg while taking  $\leq 3$  antihypertensive medications or BP  $< 140/90$  mm Hg while taking  $\geq 4$  medications. Analyses included 1,359 Chronic Renal Insufficiency Cohort (CRIC) Study participants with ATRH and 2,008 hypertensive participants without. Logistic regression was used to examine cross-sectional associations of inflammatory biomarkers and ATRH adjusting for demographic, lifestyle, and clinical risk factors and treatments. Cox proportional hazards models were used to assess the impact of inflammatory biomarkers on associations of ATRH with composite CVD and mortality beyond conventional risk factors. Multivariable-adjusted odds ratio (95% confidence intervals [CI]) of ATRH for the highest tertile vs. lowest tertile of inflammatory biomarker levels was 1.29 (95% CI, 1.05–1.59) for interleukin-6 (IL-6), 1.49 (95% CI, 1.20–1.85) for tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and 0.77 (95% CI, 0.63–0.95) for transforming growth factor- $\beta$  (TGF- $\beta$ ). High-sensitivity C-reactive protein, fibrinogen, interleukin-1 $\beta$ , and interleukin-1 receptor antagonist were not significantly associated with ATRH. Adding inflammatory biomarkers to Cox models did not attenuate the significant association of ATRH with CVD and mortality. Our findings show higher levels of IL-6 and TNF- $\alpha$  and lower levels of TGF- $\beta$  were independently associated with odds of ATRH. Targeting specific inflammatory pathways may improve BP control in CKD patients.

## Keywords

Inflammation; hypertension; resistant hypertension; chronic kidney disease; cardiovascular disease; mortality

## Introduction

The prevalence of apparent treatment-resistant hypertension (ATRH) is 40.4% in chronic kidney disease (CKD) patients compared with 8.9% in the general population in the US.<sup>1,2</sup> ATRH is significantly associated with increased risk of cardiovascular disease (CVD), death, and CKD progression in patients with CKD.<sup>1</sup> However, the etiology for exceptionally high prevalence of ATRH in CKD is not clear.

Inflammation may play a role in the etiology of hypertension.<sup>3–8</sup> In animal studies, mice deficient in interleukin-6 (IL-6),<sup>9</sup> tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ),<sup>10</sup> or interleukin-17 (IL-17),<sup>11</sup> have a lower blood pressure in response to a hypertensive dose of angiotensin (Ang) II compared with control mice. Suppression of NF- $\kappa$ B reportedly inhibits the increase in blood pressure that normally occurs in spontaneously hypertensive rats.<sup>12</sup> A prospective study also suggested that inflammatory biomarkers (IL-6 and C-reactive protein [CRP]) predict incidence of hypertension in middle-aged and older subjects without CVD enrolled in the Multi-Ethnic Study of Atherosclerosis (MESA).<sup>13</sup> Furthermore, inflammatory biomarkers (CRP, IL-6, TNF- $\alpha$ , and intercellular adhesion molecule 1 [ICAM-1]) predict CVD outcomes in prospective studies independent of hypertension and other traditional risk factors.<sup>14–17</sup> Inflammation was found to be increased in CKD patients.<sup>18–21</sup> However, it is unknown if inflammation is associated with ATRH and its related clinical outcomes that

may require specific treatment approaches. The objective of this study is to determine if inflammatory biomarkers are associated with ATRH, CVD outcomes and death among CKD patients in the Chronic Renal Insufficiency Cohort (CRIC) Study.<sup>22</sup>

## Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### Study Participants

The CRIC Study includes a racially and ethnically diverse group of men and women aged 21 to 74 years with mild-to-moderate CKD (age-based eGFR entry criteria 20–70 mL/min/1.73m<sup>2</sup>).<sup>22</sup> In total, 3,939 CRIC participants were recruited between May 2003 and August 2008 from seven clinical centers in the US. Participants were identified through searches of laboratory databases, medical records, and referrals from health care providers. Patients with cirrhosis, HIV infection, polycystic kidney disease, or renal cell carcinoma; those on dialysis or recipients of a kidney transplant; or those taking immunosuppressant drugs for glomerulonephritis were excluded from the CRIC Study. For the current analysis, those without blood pressure (BP) information (n=1), medication information (n=28), or a diagnosis of hypertension (systolic BP [SBP] <140 mm Hg and/or diastolic BP [DBP] <90 mm Hg and not taking antihypertensive medications) at baseline (n=543) were excluded, yielding a total analysis sample size of 3367. The CRIC Study was approved by the Institutional Review Boards from each participating institute. Written informed consent was obtained from all participants. The CRIC Study also conformed to the Health Insurance Portability and Accountability Act (HIPAA) guidelines.

### Data Collection

All CRIC study data were collected by trained study staff during the baseline and annual clinical visits. All data collection procedures and equipment were standardized across study sites. Baseline information on demographic characteristics, lifestyle risk factors, previous history of CVD, and use of medications was obtained by standard questionnaire. Cigarette smokers were defined as participants who smoked more than 100 cigarettes in their lifetime and reported currently smoking, and alcohol drinkers as those who consumed one or more alcoholic beverages each week over the previous year. Physical activity was calculated as total metabolic equivalents [METs] per week. Body weight and height were measured and body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Three seated blood pressure (BP) measurements were obtained by trained and certified staff after at least 5 minutes of quiet rest following a standard protocol using an aneroid sphygmomanometer, with the averages of 3 measurements used for analysis.<sup>1</sup> Hypertension was defined as systolic BP ≥140 mmHg and/or diastolic BP ≥90 mmHg and/or current use of antihypertensive medication. Diabetes was defined as a fasting plasma glucose ≥126 mg/dL, a non-fasting plasma glucose ≥200 mg/dL, or self-reported use of anti-diabetes medication.

Glucose, cholesterol, triglycerides, glycated hemoglobin (HbA1c), and total parathyroid hormone (PTH) were measured using standard laboratory methods. Fibroblast growth factor 23 (FGF23) was measured using a second-generation C-terminal assay (Immutopics, San Clemente, CA). Estimated-glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation after calibrating serum creatinine measurements to isotope dilution mass spectrometry-traceable values.<sup>23</sup> A 24-hour urine specimen was collected and urinary protein concentration was determined using the turbidometric method with benzethonium chloride. All laboratory measurements were conducted at the CRIC Study central laboratory at the University of Pennsylvania with stringent quality control.

### Definition of ATRH

ATRH was defined as mean SBP  $\geq$  140 mm Hg or mean DBP  $\geq$  90 mm Hg while taking  $\geq$  3 antihypertensive medications or mean SBP  $<$ 140 mm Hg and mean DBP  $<$ 90 mm Hg while taking  $\geq$  4 antihypertensive medications at the baseline visit. Additionally, we conducted two sensitivity analyses: the first specifying the use of a diuretic as a requirement for diagnosis of ATRH; and the second defining ATRH as mean SBP  $\geq$  130 mm Hg or mean DBP  $\geq$  80 mm Hg while taking  $\geq$  3 antihypertensive medications or taking  $\geq$  4 antihypertensive medications with mean SBP  $<$ 130 mm Hg and mean DBP  $<$ 80 mm Hg with one of the agents being a diuretic, according to the 2017 American College of Cardiology/American Heart Association (ACC/AHA) hypertension guideline.

### Measurement of Inflammatory Biomarkers

High-sensitivity sandwich ELISAs (Quantikine HS; R&D Systems, Minneapolis, MN) were used to measure plasma IL-1 $\beta$ , IL-6, and TNF- $\alpha$  levels. Standard sandwich ELISAs (Quantikine; R&D Systems) were used to quantify IL-1 receptor antagonist (IL-1RA) and transforming growth factor beta (TGF- $\beta$ ) levels. The lower detection limits for IL-1  $\beta$ , IL-6, TNF- $\alpha$ , IL-RA, and TGF-  $\beta$  were 0.06 pg/ml, 0.07 pg/ml, 0.11 pg/ml, 6.3 pg/ml, and 4.6 pg/ml, respectively. Integrated performance of IL-1  $\beta$ , IL1RA, IL-6, and TNF- $\alpha$ . ELISAs were implemented using a robotic liquid handling platform (Biomek FXp; Beckman Coulter, Brea, CA). The samples were stored at  $-80^{\circ}\text{C}$  and assays performed at the time of initial thawing to prevent degradation.<sup>24</sup> All cytokine assays were performed in duplicates using the blood samples collected at the baseline visit. Several blood samples had a concentration of IL-1 $\beta$  below the minimal level for detection; we arbitrarily assigned a very low value for IL-1 $\beta$  (0.00001) to these samples. The coefficient of variation was 13% for all cytokines assays except for TNF- $\alpha$  and TGF- $\beta$ , for which the estimated imprecision was 15.2% and 21.5%, respectively. Hs-CRP and fibrinogen were quantified in EDTA plasma samples using specific laser-based immunonephelometric methods on the BNII (Siemens Healthcare Diagnostics, Deerfield, IL). The imprecision for hs-CRP and fibrinogen were 5%. Limits of detection for hs-CRP and fibrinogen analyses were 0.16 mg/L and 0.15 g/L, respectively.

### Calculation of Inflammation Score

An inflammation score has been shown to more accurately predict the phenotype of interest than a single biomarker of inflammation.<sup>25–27</sup> In these studies, inflammation was determined to be present in a particular participant if serum level of an inflammatory biomarker exceeded its median value for the whole cohort. We computed a composite score

ranging from 0 to 5 based on levels of the following biomarkers and the value at or above which a score of 1 was assigned: hs-CRP .3 mg/L,<sup>28</sup> fibrinogen 350 mg/dl,<sup>29</sup> IL-6 6 pg/ml,<sup>30</sup> TNF- $\alpha$  7 pg/ml, and IL-1 $\beta$  0.39 pg/ml.<sup>30,31</sup> The cut-off values for individual biomarkers were chosen from published literature.

### Clinical Outcome Measures

CRIC Study participants attended annual follow-up clinic visits with interim telephone contact at 6 months. CVD outcomes were assessed using a standard Medical Event Questionnaire at all follow-up contacts. Medical records were requested for event verification. Cardiovascular events were adjudicated by blinded reviewers using predefined criteria. In the current analysis, the median duration of follow-up was 7.3 years for CVD events. Deaths were ascertained from reports by next of kin, death certificates, hospital records, and linkage with the Social Security Death Master File. In the current analysis, the median duration of follow-up was 8.4 years for death events. Details of the process of event ascertainment and adjudication in the CRIC study have been previously published.<sup>32</sup> The following incident outcomes were defined a priori for this analysis: (1) composite of congestive heart failure (CHF), myocardial infarction (MI), stroke, and peripheral arterial disease (PAD), which comprised cardiovascular disease, (2) all-cause mortality.

### Statistical Methods

Baseline characteristics of participants were summarized as means (standard deviation) for continuous variables, percentages for categorical variables, or medians (interquartile range) for variables with skewed distribution, by ATRH status. Statistical significance was tested using ANOVA for continuous variables and  $\chi^2$  tests for categorical variables. Logarithmic transformations were performed for severely skewed variables to stabilize variances and normalize distributions.

Logistic regression models were used to explore the cross-sectional association of the levels of inflammatory biomarkers and odds of ATRH by tertiles or one standard deviation/one inflammation score increment of the biomarkers. The logistic regression models include (1) age, sex, race/ethnicity, and clinic site, and (2) age, sex, race/ethnicity, clinic site, body mass index, physical activity, weekly drinking, urinary sodium, estimated glomerular filtration rate, use of statin medications, and use of angiotensin converting enzyme inhibitor (ACE) inhibitor or angiotensin receptor blocker (ARB). Hazard ratios for the associations of ATRH with composite CVD and total mortality were estimated using Cox proportional hazards models. The assumption of proportionality was tested using Schoenfeld residuals and interaction terms with time for each exposure variable and covariate. No substantial deviations from proportionality were observed. We evaluated the impact of each inflammatory biomarker and combinations of biomarkers on the association between ATRH and CVD (mortality) using sequential model adjustment as follows: (1) age, gender, race, and clinical sites; (2) age, gender, race, clinical sites, history of cardiovascular disease, total cholesterol, HDL cholesterol, systolic BP, use of antihypertensive medications, current smoking, diabetes, estimated glomerular filtration rate, 24-hour urinary protein, FGF-23, and PTH; (3) all the factors listed in (2) plus individual inflammatory biomarkers, separately; (4) all the factors listed in (2) plus IL-6, TNF- $\alpha$ , and TGF- $\beta$ ; and (5) all the factors listed in (2)

plus the inflammation score. All analyses were conducted using SAS version 9.4 (SAS Institute, Inc) and R version 3.4.2 (The R Foundation). All P values were 2-sided, and statistical significance was defined as  $P < 0.05$ .

## Results

The baseline characteristics of 1359 CRIC Study participants with ATRH and 2008 without are detailed in Table 1. Those with ATRH were older, male, black, and had less education, physical activity, alcohol intake, history of cardiovascular disease (MI, stroke, CHF, and PAD), and diabetes mellitus. Renin–angiotensin system blockers,  $\beta$  blockers, calcium channel blockers, and diuretics were more commonly used among patients with ATRH compared to those without. On average, participants with ATRH had higher BMI, SBP, total parathyroid hormone, fibroblast growth factor-23 (FGF-23), and 24-hour urinary protein, but lower HDL-cholesterol, LDL-cholesterol, and eGFR. Hs-CRP, IL-6, IL-1 $\beta$ , IL-1RA, TGF- $\beta$ , and inflammation score were not significantly different between ATRH patients who had a BP  $\geq 140/90$  mmHg on  $\geq 3$  antihypertensive medications and those who had BP  $< 140/90$  mmHg on  $\geq 4$  antihypertensive medications. However, fibrinogen and TNF- $\alpha$  were slightly higher in patients who had a BP  $\geq 140/90$  mmHg on  $\geq 3$  antihypertensive medications (Supplemental Table S1).

Odds ratios (95% confidence intervals [CI]) for ATRH associated with levels of inflammatory biomarkers as categorized tertiles or continuous one SD increase (or one unit higher of inflammatory scores) are presented in Table 2. In multivariable-adjusted models, the odds ratios for ATRH comparing the middle and highest tertile to the lowest tertile were 1.32 (95% confidence interval [CI], 1.08–1.61) and 1.29 (95% CI, 1.05–1.59) for IL-6; 1.33 (95% CI 1.09–1.63) and 1.49 (95% CI, 1.20–1.85) for TNF- $\alpha$ ; and 0.85 (95% CI, 0.70–1.04) and 0.77 (95% CI, 0.63–0.95) for TGF- $\beta$ . Similarly, the odds ratios for ATRH associated with one SD increase of log-transformed inflammatory biomarkers were 1.09 (95% CI, 1.00–1.19) for IL-6; 1.12 (95% CI, 1.03–1.22) for TNF- $\alpha$ , and 0.88 (95% CI, 0.81–0.96) for TGF- $\beta$ , respectively. The areas under the ROC curves for ATRH prevalence were 0.582, 0.566, and 0.532 for IL-6, TNF- $\alpha$ , and TGF- $\beta$ , respectively. HsCRP, fibrinogen, IL-1 $\beta$ , IL-1RA, and inflammation scores were not significantly associated with odds of ATRH after multiple adjustment.

We observed similar patterns in the association of inflammation with ATRH using alternative diagnostic criteria. In a sensitivity analysis specifying the use of a diuretic as a requirement for diagnosis of ATRH, 1215 CRIC Study participants had ATRH and 2152 participants did not. The odds ratios of ATRH for the middle and highest tertile comparing to the lowest tertile were 1.20 (95% CI, 0.98–1.48) and 1.27 (95% CI, 1.03–1.57) for IL-6; 1.25 (95% CI, 1.02–1.53) and 1.34 (95% CI, 1.08–1.67) for TNF- $\alpha$ ; and 0.94 (95% CI, 0.77–1.15) and 0.79 (95% CI, 0.64–0.97) for TGF- $\beta$ , respectively. The odds ratios for ATRH associated with one SD increase of log-transformed inflammatory biomarkers were 1.09 (95% CI, 1.00–1.18) for IL-6; 1.09 (95% CI, 1.00–1.19) for TNF- $\alpha$ , and 0.88 (95% CI, 0.81–0.96) for TGF- $\beta$  (Supplemental Table S2), respectively. In another sensitivity analysis defining ATRH according to the 2017 ACC/AHA hypertension guideline criteria, 1536 CRIC Study participants had ATRH and 1831 participants did not. The odds ratios of ATRH

for the middle and highest tertile comparing to the lowest tertile were 1.25 (95% CI, 1.03–1.52) and 1.31 (95% CI, 1.07–1.61) for IL-6; 1.26 (95% CI, 1.04–1.53) and 1.48 (95% CI, 1.20–1.82) for TNF- $\alpha$ ; and 0.92 (95% CI, 0.76–1.12) and 0.88 (95% CI, 0.72–1.07) for TGF- $\beta$ , respectively. The odds ratios for ATRH associated with one SD increase of log-transformed inflammatory biomarkers were 1.11 (95% CI, 1.02–1.21) for IL-6; 1.10 (95% CI, 1.02–1.20) for TNF- $\alpha$ , and 0.94 (95% CI, 0.86–1.02) for TGF- $\beta$  (Supplemental Table S3), respectively. Finally, the odds ratios of ATRH associated with inflammatory biomarkers did not significantly differ in obese and non-obese patients (Supplemental Table S4).

The impact of inflammatory biomarkers on the associations between ATRH and CVD and all-cause mortality are presented in Table 3. The hazard ratios (HR) associated with ATRH are 1.49 (95% CI, 1.29–1.73) for CVD and 1.27 (95% CI, 1.09–1.48) for death after adjusting for age, sex, race/ethnicity, clinical site, history of cardiovascular disease, total cholesterol, HDL cholesterol, systolic BP, use of antihypertensive medications, current cigarette smoking, diabetes, estimated-glomerular filtration rate, 24-hour urinary protein, FGF23, and PTH. Adding inflammatory biomarkers to the multivariate models did not change the hazard ratios of CVD and mortality associated with ATRH. Similar findings were seen while using an alternative diagnostic criteria with a diuretic as a required agent for diagnosis of ATRH or the 2017 ACC/AHA guideline diagnostic criteria for resistant hypertension. (Supplemental Tables S5 and S6).

## Discussion

The present study indicates that specific inflammatory biomarkers including IL-6, TNF- $\alpha$ , and TGF- $\beta$  are significantly associated with odds of ATRH, independent of current use of medications with anti-inflammatory effects, such as ACE I or ARB, and statins. However, the levels of other inflammatory biomarkers, including hsCRP, fibrinogen, IL-1 $\beta$ , and IL-1 receptor antagonist, were not significantly associated with odds of ATRH. In addition, inflammation may not explain the excess risk of CVD and mortality among CKD patients with ATRH beyond severity of this condition. Similar findings were seen using the 2017 ACC/AHA guideline diagnostic criteria for resistant hypertension.

These findings have important clinical and public health implications because ATRH is highly prevalent and associated with significant risk CVD and mortality among patients with CKD. Understanding the potential etiology for significantly high risk of ATRH and associated complications among CKD patients may guide discovery of novel and more effective treatments for reducing ATRH and its complications. Individuals with ATRH require more than three to four antihypertensive drugs to control BP and the side effects and costs associated with treatment are substantial. Therefore, targeting specific or potential upstream etiology may increase efficacy of treatment and reduce unnecessary side effects and cost.

Although inflammation may play a role in the etiology of hypertension,<sup>3-8</sup> it is unclear whether inflammation is associated with exceptionally high prevalence of ATRH in CKD patients. In animal studies, mice deficient in IL-6<sup>9</sup> and TNF- $\pm$ <sup>10</sup> have a lower blood pressure in response to a hypertensive dose of angiotensin (Ang) II compared with control mice;



RNA interference knockdown of IL-6 in rats has also been shown to significantly attenuate cold-induced elevation of systolic BP,<sup>33</sup> suggesting inflammation may aggregate BP in the presence of additional BP stimuli. In human studies, prospective studies also suggested that inflammatory biomarkers IL-6 and CRP predict incidence of hypertension in middle-aged and older subjects without CVD enrolled in the Multi-Ethnic Study of Atherosclerosis (MESA),<sup>13</sup> suggesting inflammation may be part of the upstream etiology of hypertension. Inflammatory biomarkers IL-6 and TNF- $\alpha$  were found to be elevated in CKD patients and inversely associated with eGFR<sup>19,21</sup>. In addition, a previous study documented that the prevalence of ATRH is much higher among patients with low eGFR (54% for those with eGFR<30 ml/min/1.73 m<sup>2</sup> vs. 39% for those with eGFR 30–60 ml/min/1.73 m<sup>2</sup>).<sup>1</sup> These previous findings along with our results suggest specific inflammatory pathways involving IL-6 and TNF- $\alpha$  may be associated with increased risk of ATRH in CKD, independent of potential anti-inflammation effect of ACE I, ARB and statin treatment. More specific anti-inflammatory agents may potentially improve BP control.

Our study is the first to report the inverse association of TGF- $\beta$  with ATRH. TGF- $\beta$  level was found to be lower among patients with lower eGFR.<sup>21</sup> Even though the relationship of TGF- $\beta$  and BP has not been reported, TGF- $\beta$  inhibits IL-1 $\beta$  production and CD4+ and CD8+ lymphocyte activation and therefore inhibits TNF- $\alpha$  and IL-17 production.<sup>33,34</sup> IL-6 is needed for polarization of TH17 cells, and mice lacking this cytokine have lower BP and less endothelial dysfunction in response to Ang II infusion.<sup>35,36</sup> Normalizing upstream factor TGF- $\beta$ , thereby lowering IL-6 and TNF- $\alpha$ , may improve resistant hypertension or hypertension control and warrants further investigation.

Hs-CRP levels have been shown to predict the risk of hypertension among the general population in several cohort studies.<sup>37–39</sup> However, this association has not been established in patients with CKD. In addition, it has been suggested that antihypertensive medications, such as ACEI and ARB, could reduce CRP levels independent of their blood-pressure lowering effect.<sup>40</sup> In the current study, a majority of participants were on antihypertensive medications, which might explain the lack of association between CRP and ATRH.

Prospective studies have suggested that inflammatory biomarkers such as hsCRP, IL-6, and TNF- $\alpha$  are associated with CVD.<sup>14–17</sup> In the present analysis, adjusting for those and other inflammatory biomarkers or inflammatory score in addition to traditional risk factors in multivariable models did not alter the association of ATRH with CVD and mortality, suggesting that the association of ATRH with CVD and all-cause mortality is independent of both traditional risk factors and inflammation. One potential explanation is that inflammation may not explain CVD and death risk related with ATRH beyond traditional risk factors. Another possibility is that inflammation may be an upstream etiological factor of ATRH and vascular damage, which may not be reversible or altered by modification of traditional risk factors and inflammation. Therefore, preventing ATRH, potentially via inflammation-focused strategies, is key to reducing its related adverse clinical outcomes.

The present study has several strengths. First, this study contains a relatively large sample of CKD patients and multiple covariables were collected for analysis. Second, multiple important inflammatory biomarkers were measured for analyses. Third, CVD and mortality

outcomes are well defined and collected according to a standardized protocol, which minimizes bias. However, this study cannot establish a temporal relationship or make causality inferences due to its cross-sectional and observational nature. In addition, even though we included all patients on diuretics in the ATRH group in sensitivity analyses, we could not be sure that the maximum dose of diuretics was used. This might weaken the association by potentially misclassifying non-ATRH into ATRH. Furthermore, 24-hour ambulatory BP monitoring (ABPM) is recommended to exclude “white-coat” hypertension and confirm ATRH. We did not collect ABPM data in all CRIC study participants which could further misclassify non-ATRH into ATRH and weaken observed associations. On the other hand, poor adherence to antihypertensive medications, such as ACEI and ARB, could result in both ATRH and increased levels of inflammatory biomarkers. However, the use of ACEI and ARB were adjusted in multiple models in our analyses.

In conclusion, higher levels of IL-6 and TNF- $\alpha$  as well as lower levels of TGF- $\beta$  (a potential anti-inflammatory biomarker) were associated with odds of ATRH independent of current use of potentially anti-inflammatory agents including ACE I, ABRB and statin treatment.

### Perspectives

This study provides the first evidence that higher levels of IL-6 and TNF- $\alpha$  and lower levels of TGF- $\beta$  (as an anti-inflammatory factor), but not overall inflammation, were independently associated with ATRH in CKD. Further research is warranted to explore the temporal relationship of inflammation and ATRH as well as if targeting specific inflammation pathways may potentially increase effectiveness in prevention and control of ATRH in CKD and ultimately reduce its related adverse outcomes.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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**Novelty and Significance****What Is New?**

The etiology for exceptionally high prevalence of apparent treatment resistant hypertension (ATRH) in chronic kidney disease (CKD), despite high utilization of diuretics, is not clear. Our study is the first to evaluate inflammation and ATRH in CKD. This study provides the first evidence that higher levels of IL-6 and TNF- $\alpha$  and lower levels of TGF- $\beta$  (as an anti-inflammatory factor), but not overall inflammation, were independently associated with ATRH in CKD.

**What Is Relevant?**

The prevalence of ATRH is much higher in CKD patients compared with the general population. ATRH is significantly associated with increased risk of cardiovascular disease, death, and CKD progression in patients with CKD. Prevention and control of ATRH is key to reduce ATRH-associated adverse outcomes. However, the etiology for exceptionally high prevalence of ATRH in CKD is not clear. Our study findings suggest that specific inflammatory pathways may be involved in etiology of ATRH.

**Summary**

Higher levels of IL-6 and TNF- $\alpha$  as well as lower levels of TGF- $\beta$  (a potential anti-inflammatory biomarker) were associated with odds of ATRH independent of current use of potentially anti-inflammatory agents including ACE I, ABRB and statin treatment. Further study is warranted to investigate if targeting specific inflammatory pathways could improve blood pressure control and prevent ATRH among patients with CKD.

**Table 1.** Baseline Characteristics of the Study Participants by Apparent Treatment-Resistant Hypertension Status

Variables	No Apparent Treatment Resistant Hypertension (n=2008)	Apparent Treatment Resistant Hypertension (n=1359)	P Value
Age, mean (SD), years	57.4 (11.2)	60.1 (9.2)	<0.001
Male, no. (%)	1082 (53.9)	798 (58.7)	0.006
Race/ethnicity, no. (%)			
Non-Hispanic white	896 (44.6)	388 (28.6)	
Non-Hispanic black	776 (38.6)	747 (55.0)	<0.001
Hispanic	266 (13.2)	173 (12.7)	
Other	70 (3.5)	51 (3.8)	
High school education, no. (%)	1597 (79.5)	997 (73.4)	<0.001
Physical activity, mean (SD), total MET	206.1 (152.1)	176.6 (129.2)	<0.001
Current smoking, no. (%)	278 (13.8)	169 (12.4)	0.26
Weekly drinking, no. (%)	444 (22.1)	185 (13.6)	<0.001
Diabetes mellitus, no. (%)	863 (43.0)	889 (65.4)	<0.001
History of cardiovascular disease, no. (%)	506 (25.2)	697 (51.3)	<0.001
Use of lipid-lowering medication, no. (%)	1159 (57.7)	971 (71.4)	<0.001
Use of statin medications, no. (%)	1067 (53.1)	913 (67.2)	<0.001
Use of aspirin, no. (%)	808 (40.2)	720 (53.0)	<0.001
Use of antihypertensive medications, no. (%)	1955 (97.4)	1359 (100.0)	<0.001
ACE-inhibitors	978 (48.7)	814 (59.9)	<0.001
ARBs	447 (22.3)	493 (36.3)	<0.001
ACE-inhibitors or ARBs	1361 (67.8)	1153 (84.8)	<0.001
Beta blockers	720 (35.9)	1114 (82.0)	<0.001
Calcium channel blockers	618 (30.8)	939 (69.1)	<0.001
Diuretics	994 (49.5)	1215 (89.4)	<0.001
Body-mass index, mean (SD), kg/m <sup>2</sup>	31.6 (7.7)	33.7 (7.8)	<0.001
Systolic blood pressure, mean (SD), mm Hg	125.3 (18.7)	139.5 (24.0)	<0.001
Diastolic blood pressure, mean (SD), mm Hg	71.9 (12.1)	72.4 (14.5)	0.28
Total cholesterol, mean (SD), mg/dL	185.8 (45.9)	178.9 (45.9)	<0.001

Variables	No Apparent Treatment Resistant Hypertension (n=2008)	Apparent Treatment Resistant Hypertension (n=1359)	P Value
HDL cholesterol, mean (SD), mg/dL	48.3 (15.8)	45.3 (14.2)	<0.001
LDL cholesterol, mean (SD), mg/dL	104.0 (34.9)	98.7 (35.8)	<0.001
Parathyroid hormone, median (IQR), pg/mL	50.0 (33.1, 80.1)	70.00 (44.6, 115.4)	<0.001
Fibroblast growth factor-23, median (IQR), RU/mL	136.2 (94.1, 219.1)	179.1 (115.9, 293.2)	<0.001
Urinary sodium, mean (SD), mmol/L	161.7 (77.6)	164.9 (77.8)	0.25
Estimated GFR, mean (SD), ml/min/1.73 m <sup>2</sup>	45.1 (14.2)	39.4 (13.0)	<0.001
Urinary protein, median (IQR), g/24 hours	0.14 (0.07, 0.70)	0.41 (0.10, 1.66)	<0.001
High-sensitive C-reactive protein, median (IQR), mg/L	2.62 (1.08, 6.60)	2.90 (1.16, 7.05)	0.07
Fibrinogen, mean (SD), mg/dL	4.12 (1.18)	4.43 (1.25)	<0.001
Interleukin-6, median (IQR), pg/mL	1.83 (1.14, 3.04)	2.24 (1.46, 3.55)	<0.001
Tumor necrosis factor- $\alpha$ , median (IQR), mg/dL	2.20 (1.50, 3.20)	2.50 (1.70, 3.50)	<0.001
Interleukin-1 $\beta$ , median (IQR), pg/mL	0.19 (0.06, 1.27)	0.29 (0.06, 1.48)	0.01
Interleukin-1 receptor antagonist, median (IQR), pg/mL	703.0 (391.0, 1513.1)	804.6 (435.5, 1683.8)	0.001
Transforming growth factor- $\beta$ , median (IQR), ng/mL	11.63 (6.97, 18.29)	10.40 (6.45, 17.07)	0.002
Inflammation score, no. (%)			
0	281 (14.3)	115 (8.7)	
1	568 (28.9)	346 (26.2)	
2	660 (33.6)	500 (37.9)	
3	365 (18.6)	277 (21.0)	<0.001
4	84 (4.3)	72 (5.5)	
5	6 (0.3)	11 (0.8)	

SD = standard deviation; MET = metabolic equivalent; ACE = angiotensin converting enzyme; ARB = angiotensin receptor blocker; HDL = high-density lipoprotein; LDL = low-density lipoprotein; IQR = inter quartile range; GFR = glomerular filtration rate.



Odds Ratios (95% Confidence Intervals) for Apparent Treatment-resistant Hypertension Associated with Levels of Inflammatory Biomarkers

**Table 2.**

Inflammatory Biomarkers	Age, sex, race/ethnicity, and clinic site-adjusted*		Multivariable-adjusted*	
	OR (95% CI)	P Value	OR (95% CI)	P Value
<b>High-sensitivity C-reactive protein</b>				
Tertile 1 (<1.46 mg/L)	1 (Reference)		1 (Reference)	
Tertile 2 (1.46–4.81 mg/L)	1.04 (0.87–1.24)	0.94	0.97 (0.80–1.18)	0.25
Tertile 3 ( 4.82 mg/L)	1.02 (0.85–1.22)		0.89 (0.73–1.09)	
Per 1 SD increase (1.26 mg/L)	1.02 (0.95–1.09)	0.65	0.95 (0.87–1.03)	0.25
<b>Fibrinogen</b>				
Tertile 1 (<3.69 mg/dL)	1 (Reference)		1 (Reference)	
Tertile 2 (3.69–4.55 mg/dL)	1.22 (1.02–1.46)	<0.001	1.03 (0.84–1.25)	0.10
Tertile 3 ( 4.56 mg/dL)	1.64 (1.36–1.96)		1.18 (0.96–1.45)	
Per 1 SD increase (1.22 mg/dL)	1.23 (1.14–1.33)	<0.001	1.07 (0.99–1.17)	0.10
<b>Interleukin-6</b>				
Tertile 1 (<1.48 pg/mL)	1 (Reference)		1 (Reference)	
Tertile 2 (1.48–2.72 pg/mL)	1.59 (1.33–1.91)	<0.001	1.32 (1.08–1.61)	0.06
Tertile 3 ( 2.73 pg/mL)	1.68 (1.40–2.02)		1.29 (1.05–1.59)	
Per 1 SD increase (0.89 pg/mL)	1.19 (1.11–1.29)	<0.001	1.09 (1.00–1.19)	0.05
<b>Tumor necrosis factor-<math>\alpha</math></b>				
Tertile 1 (<1.80 mg/dL)	1 (Reference)		1 (Reference)	
Tertile 2 (1.80–2.99 mg/dL)	1.62 (1.35–1.94)	<0.001	1.33 (1.09–1.63)	0.001
Tertile 3 ( 3.00 mg/dL)	1.88 (1.55–2.27)		1.49 (1.20–1.85)	
Per 1 SD increase (0.69 mg/dL)	1.22 (1.13–1.31)	<0.001	1.12 (1.03–1.22)	0.009
<b>Interleukin-1<math>\beta</math></b>				
Tertile 1 (<0.13 pg/mL)	1 (Reference)		1 (Reference)	
Tertile 2 (0.13–0.79 pg/mL)	1.00 (0.83–1.22)	0.26	0.97 (0.79–1.20)	0.41
Tertile 3 ( 0.80 pg/mL)	1.10 (0.92–1.32)		1.07 (0.88–1.30)	

Inflammatory Biomarkers	Age, sex, race/ethnicity, and clinic site-adjusted*		Multivariable-adjusted*	
	OR (95% CI)	P Value	OR (95% CI)	P Value
Per 1 SD increase (1.72 pg/mL)	1.05 (0.97–1.14)	0.22	1.04 (0.95–1.13)	0.39
Interleukin-1 receptor antagonist				
Tertile 1 (<490.6 pg/mL)	1 (Reference)		1 (Reference)	
Tertile 2 (490.6–1216.0 pg/mL)	1.31 (1.10–1.57)	0.004	1.17 (0.96–1.42)	0.16
Tertile 3 ( 1216.1 pg/mL)	1.39 (1.16–1.68)		1.20 (0.98–1.48)	
Per 1 SD increase (0.98 pg/mL)	1.11 (1.03–1.20)	0.006	1.05 (0.97–1.14)	0.25
Transforming growth factor-β				
Tertile 1 (<8.03 ng/mL)	1 (Reference)		1 (Reference)	
Tertile 2 (8.03–15.06 ng/mL)	0.80 (0.67–0.96)	<0.001	0.85 (0.70–1.04)	0.02
Tertile 3 ( 15.07 ng/mL)	0.68 (0.56–0.82)		0.77 (0.63–0.95)	
Per 1 SD increase (0.67 ng/mL)	0.85 (0.78–0.92)	<0.001	0.88 (0.81–0.96)	0.004
Inflammation Score				
1	1.35 (1.04–1.76)	0.001	1.10 (0.83–1.47)	0.39
2	1.59 (1.23–2.07)		1.18 (0.89–1.57)	
3	1.52 (1.15–2.03)		1.10 (0.80–1.50)	
4	1.61 (1.07–2.40)		1.10 (0.70–1.72)	
5	3.51 (1.23–10.01)		3.43 (1.04–11.29)	
Per unit increase	1.12 (1.05–1.21)	0.001	1.04 (0.96–1.12)	0.39

\* Adjusted for age, sex, race/ethnicity, clinic site, body mass index, physical activity, weekly alcohol drinking, 24-hour urinary sodium excretion, estimated-glomerular filtration rate, use of statin medications, and use of ACE inhibitors or ARBs.

**Table 3.**

Hazard Ratios (95% Confidence Intervals) for Composite Cardiovascular Disease and All-cause Mortality Associated with Apparent Treatment-Resistant Hypertension Adjusting for Inflammatory Biomarkers

Model	Composite CVD			All-cause mortality		
	HR (95% CI)	P Value	HR (95% CI)	P Value	P Value	
Model 1	2.21 (1.94–2.51)	<0.001	1.79 (1.57–2.04)	<0.001	<0.001	
Model 2	1.49 (1.29–1.73)	<0.001	1.27 (1.09–1.48)	0.002	0.002	
Model 3						
+ High-sensitivity C-reactive Protein	1.49 (1.29–1.73)	<0.001	1.28 (1.10–1.49)	0.002	0.002	
+ Fibrinogen	1.49 (1.29–1.73)	<0.001	1.27 (1.09–1.48)	0.002	0.002	
+ Interleukin-6	1.49 (1.28–1.72)	<0.001	1.27 (1.09–1.48)	0.002	0.002	
+ Tumor Necrosis Factor- $\alpha$	1.50 (1.30–1.74)	<0.001	1.27 (1.09–1.48)	0.002	0.002	
+ Interleukin-1 $\beta$	1.50 (1.29–1.73)	<0.001	1.27 (1.09–1.48)	0.002	0.002	
+ Interleukin-1 receptor antagonist	1.49 (1.29–1.73)	<0.001	1.27 (1.09–1.48)	0.002	0.002	
+ Transforming growth factor- $\beta$	1.49 (1.29–1.73)	<0.001	1.27 (1.09–1.48)	0.002	0.002	
Model 4	1.49 (1.28–1.73)	<0.001	1.28 (1.10–1.49)	0.001	0.001	
Model 5	1.49 (1.29–1.73)	<0.001	1.27 (1.09–1.48)	0.002	0.002	

Model 1: Adjusted for age, sex, race/ethnicity, and clinical site; Model 2: Model 1 + history of cardiovascular disease, total cholesterol, HDL cholesterol, systolic BP, use of antihypertensive medications, current cigarette smoking, diabetes, estimated-glomerular filtration rate, 24-hour urinary protein, fibroblast growth factor-23, and PTH; Model 3: Model 2 + individual inflammatory biomarkers; Model 4: Model 2 + interleukin-6, tumor necrosis factor- $\alpha$ , and transforming growth factor- $\beta$ ; Model 5: Model 2 + inflammation score.