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Association between Circulating Specific Leukocyte Types and Incident Chronic Kidney Disease: the Atherosclerosis Risk in Communities (ARIC) Study

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Abstract

Progressive renal fibrosis is a characteristic of all the diseases that cause renal failure and is invariably accompanied by a prominent leukocyte infiltration in the kidney. The goal of this study was to determine the association between the circulating specific leukocyte types, and incident chronic kidney disease (CKD). In a cohort of 10,056 middle aged white and African-American adults, levels of circulating neutrophils, lymphocytes and monocytes were measured at baseline; Blood pressure (BP) and serum creatinine were measured and estimated glomerular filtration rate (eGFR) was calculated at baseline and 3 and 9 years later; and surveillance for first hospitalization or death with CKD was carried out over a mean follow-up of 7.4 years (maximum 11.9 years). Increased neutrophil levels and decreased lymphocyte levels were significantly associated with greater CKD incidence after adjustment for covariates. African-Americans tended to have similar but stronger patterns of association between circulating leukocytes and CKD incidence than whites, although the differences between race groups were not statistically significant. We also found that eGFR and BPs were higher at each visit in African-Americans than whites between age 45–65. These findings support a potential role for circulating specific leukocytes in the pathogenesis of kidney dysfunction, especially in African-Americans indicating the leukocyte-related renal mechanism of essential hypertension (HT).

Keywords

Neutrophil; Lymphocyte; Glomerular hyperfiltration; Kidney dysfunction

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INTRODUCTION

In population studies the most important risk factors for the development and progression of chronic kidney disease (CKD) are diabetes, hypertension, and vascular disease.^{1,2} However, these factors do not entirely explain the variation in the population incidence and prevalence of CKD.²

Systemic inflammation and CKD are frequently associated.³ Levels of circulating inflammatory biomarkers are elevated in elderly individuals with mild to moderate renal dysfunction⁴ and in an otherwise healthy population with early kidney damage.⁵ In patients with established CKD, higher levels of inflammatory markers were associated with faster progression of CKD.⁶ Among those markers, total white blood cell (WBC) count has been reported to predict kidney function decrease.^{7,8,9} However, the association between specific types of circulating leukocytes and the incidence of CKD is unknown.

A characteristic feature of renal failure is progressive renal fibrosis, invariably associated with a prominent leukocyte infiltrate consisting of macrophages and T cells, together with apoptosis of tubular epithelial cells and destruction of peritubular capillaries.¹⁰ Studies in animal models suggest that inflammation contributes to afferent arterial damage and consequent glomerular hyperfiltration and renal damage.^{11,12} We hypothesized that circulating specific leukocyte types could be associated with kidney damage and/or renal dysfunction and that the mechanism could involve inflammation-related glomerular hyperfiltration.

In the general population, African-Americans are four times as likely as whites to develop kidney failure,¹³ and hypertension-associated renal damage is 4–6 times more common in African-Americans than whites.^{14,15,16} Hence, the above hypothesis may be especially applicable to African-Americans. Therefore, the major goals of this study were to determine whether there is an independent association in African-Americans and whites between circulating specific leukocyte types (neutrophils, lymphocytes and monocytes) and the incidence of CKD in a middle age population and whether this association differs between African-Americans and whites. In addition, to explore the possible mechanism of leukocyte-related CKD, the longitudinal association between BP and estimated glomerular filtration rate (eGFR) in African-Americans and whites was examined.

MATERIALS AND METHODS

Study population, exclusions and study design

The ARIC study is a prospective investigation of atherosclerosis and clinical atherosclerotic diseases in four US communities. A population sample totaling 15,792 persons aged 45–64 was selected from Forsyth County, NC, Jackson, MS, the northwest suburbs of Minneapolis, MN, and Washington County, MD, using a published study design, sampling strategy, and examination techniques.¹⁷ Each community's cohort was a probability sample, except that only African-Americans were sampled in Jackson. A baseline examination (visit 1) was conducted from 1986 to 1989, with response rates of 46% in Jackson and 65–67% in the other three communities. Participants were re-examined every three years (visit 2, 1990–92; visit 3, 1993–95; visit 4, 1996–98). At each clinic visit, sociodemographic characteristics, medical history, medication use, diet, and physical activity were assessed, and a variety of biochemical, physiologic, and anthropomorphic measures were obtained, including leukocyte levels at baseline (visit 1) and BP at each visit. Serum creatinine was measured at visits 1, 2 and 4.

For this study, we excluded (numbers not mutually exclusive) 1,432 participants with prevalent CKD at baseline (eGFR < 60 mL/min/1.73 m²); 210 participants with missing values for eGFR at baseline; 3,733 participants with missing leukocyte counts at baseline; 1,110 participants with prevalent chronic heart disease (CHD) or missing data on CHD status at baseline; 763 participants with missing values for covariates at baseline (BP, hypertension (HT) medications, body mass index (BMI), diabetes status, smoking/drinking status, or total cholesterol); and 103 participants who were neither white nor African-American or who were African-Americans in the Minneapolis, MN and Washington County, MD field centers (due to their small numbers). This left 10,056 participants (6,676 whites and 3,380 African-Americans) available for the main sample.

In this main study group, we examined the association between leukocyte levels at baseline (visit 1) and the development of incident CKD (defined below) through visit 4, in both whites and African-Americans. Of the 10,056 in the main sample, 7,140 (71%) had complete data on BP, eGFR, and use of HT medications for visits 1, 2 and 4; in this subgroup we examined the simultaneous change in BP and eGFR between visits 1 and 4, again in both whites and African-Americans.

Clinical definitions and measurements

All clinical measures were assessed using standardized instruments and a strict protocol. WBCs were retrieved from whole anti-coagulated blood and were determined by automated particle counters within 24 hours after venipuncture in local hospital hematology laboratories. Because there are well-known differences between whites and African-Americans in total and differential white cell counts^{18,19}, it was decided to use percentages rather than absolute values of leukocyte types; therefore, relative neutrophil, lymphocyte and monocyte levels were measured at baseline and categorized into tertiles. Serum creatinine was measured by using a modified kinetic Jaffé reaction. Serum creatinine concentration was corrected for interlaboratory differences and calibrated to the Cleveland Clinic laboratory measurements by subtraction of 0.24 mg/dL at visits 1 (baseline) and 2 and addition of 0.18 mg/dL at visit 4 (serum creatinine was not measured at visit 3). GFR was estimated by using the 4-variable MDRD (Modification of Diet in Renal Disease) study equation developed at the Cleveland Clinic: estimated GFR (eGFR) = $186.3 \times (\text{serum creatinine [mg/dL]} - 1.154) \times (\text{age} - 0.203) \times (0.742 \text{ if female}) \times (1.21 \text{ if African American})$.²⁰ Incident CKD was defined as either: (1) an eGFR decreasing to less than 60 mL/min/1.73 m², representing stages 3 to 5 of CKD, at the 3-year (visit 2) or 9-year (visit 4) follow-up visit; or (2) a hospitalization or death with CKD.²¹ Deaths and hospitalizations were identified through annual participant interviews, local hospital discharge lists, and county death certificates and included all those coded (International Classification of Diseases, Ninth Revision [ICD-9]) for chronic renal disease (581 to 583.91 and 585 to 588.91), hypertensive renal disease (403 to 403.91), hypertensive heart and renal disease (404 to 404.93), unspecified disorder of kidney and ureter (593.9), diabetes with renal manifestations (250.40 to 250.43), kidney transplant, renal dialysis or adjustment/fitting of catheter (V42.0, V45.1, or V56), or either hemodialysis (39.95) or peritoneal dialysis (54.98) without simultaneous acute renal failure (584, 586, 788.9, and 958.5). Corresponding ICD-10 codes were used for deaths after ICD-10 implementation. The follow-up period was defined as the time elapsed from the date of the baseline visit (visit 1) to the date of the first incident event or to December 31, 1998 if no incident event was detected. Those lost to follow-up were censored.

Systolic and diastolic BP (SBP and DBP, respectively) were taken in the sitting position by trained technicians using a random-zero sphygmomanometer after a 5-minute rest, and the average of the last two values was computed. Diabetes was defined based on the American Diabetes Association guidelines as fasting serum glucose of ≥ 126 mg/dL (7 mmol/L) or

non-fasting glucose of ≥ 200 mg/dL (11.1 mmol/L), or self-reported use of diabetic medications within two weeks of the clinic visit, or a history of physician-diagnosed diabetes. BMI was calculated as weight (kg) divided by height (m) squared. Self-reported smoking and drinking status were categorized into 3 levels (current, former, never). Fasting serum total lipoprotein cholesterol concentration was assessed with Roche enzymatic methods using a Cobas centrifuge analyzer (Hoffman-La Roche), with the laboratory certified by the CDC-NHLBI Lipid Standardization Program.

Statistical Analysis

Baseline data are presented by tertile of neutrophil level as mean $\pm$ SE for continuous variables and percentages for categorical variables. Clinical characteristics by neutrophil tertile were compared using χ^2 and ANOVA F tests. (Baseline characteristics by tertile of lymphocyte level were distributed similarly and are not shown.) A two-sided p value < 0.05 was used as the cut point to assess statistical significance. Cox proportional hazards regression was used to estimate the unadjusted and covariate-adjusted hazard ratio (HR) of CKD for a 10 percentage point change in leukocyte level, and also by tertile of leukocyte using the lowest tertile as the reference group. The proportional hazards assumption was evaluated graphically; there was no evidence of violation. The interaction term between leukocyte level/tertile and baseline eGFR was statistically significant and retained in all models. All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

RESULTS

At baseline, the study population comprised 41% men and 34% African-Americans, with a mean age of 53.7 years (range 44 – 66 years, standard deviation [SD] 5.8 years); 10% had diabetes, 28% were current smokers, 57% were current drinkers, and 26% were on HT medications. The mean values for BMI and total cholesterol were 27.2 kg/m² (SD 5.2) and 5.95 mmol/L (SD 2.1), respectively. The mean SBP and DBP (in mm Hg) were 121.2 (SD 18.8) and 74.0 (SD 11.3), respectively. The mean values for white cell type levels were: neutrophils: 56.2% (SD 11.8); lymphocytes: 33.9% (SD 10.1); and monocytes: 6.0% (SD 2.7). The mean eGFR (in mL/min/1.73m²) was 90.1 (SD 21.6) at visit1; 81.7 (SD 19.0) at visit 2; and 77.6 (SD 19.1) at visit 4. There were 1,509 incident events over a mean follow-up time of 7.4 years (maximum 11.9 years), including 12 with a first hospitalization or death with CKD.

Because of the well-known differences in white blood cell counts and absolute neutrophil counts between whites and African-Americans, the clinical characteristics of the study population by tertile of neutrophil level are shown separately for whites and African-Americans in Table 1. In both groups, differences between tertiles in mean age, BMI, and total cholesterol were very small. The highest tertile had a higher mean SBP and (in African-Americans) a lower mean DBP. The highest tertile in both groups also had a higher proportion of men, diabetics, smokers, and drinkers.

Results presented from here on are limited to those for neutrophils and lymphocytes; no associations were found between monocytes and incident CKD, and these results are not shown.

CKD incidence by tertile of neutrophil and lymphocyte level

A 10 percentage point increase in the neutrophil level was associated with an HR (adjusted for baseline eGFR) of 2.0 (95% CI: 1.5, 2.7) and this was strengthened slightly (2.2 [1.6, 2.9]) after adjustment for baseline eGFR, age, sex, and race. There was no further change

after further adjustment for diabetes, BMI, smoking, drinking, and total cholesterol. Compared to the bottom tertile, the top tertile of neutrophil level was associated with a multivariable-adjusted HR of 6.3 (2.5, 15.7), and the middle tertile with a HR of 5.2 (2.2, 12.7).

A 10 percentage point increase in the lymphocyte level was associated with an HR of 0.45 (0.3, 0.6) and this changed very little after adjustment for covariates. Compared to the bottom tertile, the top tertile of lymphocyte level was associated with a multivariable-adjusted HR of 0.2 (0.1, 0.5), and the middle tertile with a multivariable-adjusted HR of 1.1 (0.4, 2.8).

Comparison of CKD incidence by tertile of neutrophil and lymphocyte level in African-Americans and whites

In African-Americans a 10 percentage point increase in the neutrophil level was associated with a multivariable-adjusted HR of 2.1 (1.3, 3.4), compared to 1.5 (0.9, 2.6) in whites. This difference was not statistically significant ($P=0.11$). In African-Americans, compared to the bottom tertile, the top tertile of neutrophil level was associated with an adjusted HR of 7.4 (1.7, 33.2), and the middle tertile with an HR of 4.6 (1.0, 20.6) (P for trend across tertiles = 0.01), compared to 2.2 (0.7, 6.9) and 0.8 (0.3, 2.3), respectively, in whites (P for trend across tertiles = 0.17) (Figure 1).

In African-Americans, a 10 percentage point increase in the lymphocyte level was associated with an adjusted HR of 0.5 (0.3, 0.9), compared to 0.6 (0.4, 1.1) in whites. This difference was not statistically significant ($P=0.35$). In African-Americans, compared to the bottom tertile, the top tertile of lymphocyte level was associated with an adjusted HR of 0.3 (0.1, 1.4), and the middle tertile with an HR of 1.3 (0.3, 6.2) (P for trend across tertiles = 0.13), compared to 0.5 (0.2, 1.7) and 0.7 (0.2, 2.3), respectively, in whites (P for trend across tertiles = 0.17) (Figure 2).

Comparison of SBP/eGFR plots in African-Americans and whites

Figure 3 shows, by race group, the vector plots of mean SBP vs mean eGFR (means for both adjusted for age, sex, diabetes, BMI, smoking, drinking, and total cholesterol) in the subgroup of participants with complete data on SBP, eGFR, and use of HT medications for visits 1, 2 and 4. (Note that values for visit 3 were obtained by interpolation, and that a constant of 10 mm Hg was added to SBP and 5 mm Hg to DBP if the participant reported being on HT medications.^{22,23}). At every visits, African-Americans had markedly higher mean values for both eGFR and SBP than whites.

DISCUSSION

Our major findings in the current study are: 1) In the ARIC population, increased circulating neutrophil levels and decreased circulating lymphocyte levels were independently associated with increased risk of incident CKD; 2) African-Americans tended to have similar but stronger patterns of association between the above circulating specific leukocyte types and CKD incidence than whites although the differences between race groups were not statistically significant. 3) At each of the 4 visits, African-Americans have both higher eGFR levels and BP than whites.

Recently, we found that increased circulating neutrophil levels and decreased circulating lymphocyte levels were significantly correlated with the regulation of BP and the development of HT, especially in African-Americans, and proposed that circulating specific leukocytes may participate in the pathogenesis of HT through a renal mechanism²⁴.

To recap briefly, by using animal models, we and others have suggested that increased renal oxidative stress^{25,26,27}, renal inflammation^{28,29}, renal local angiotensin II (Ang II) activity^{30,31} are all important in the pathogenesis of hypertension³². The combination and interaction of these detrimental renal factors, especially through the inactivation of NO bioavailability^{33,34} by oxidative stress, drives tubular sodium reabsorption^{35,36}, resulting in volume expansion, increased cardiac output, systemic vascular resistance^{37,38} and HT.

Evidence has shown that increased renal oxidative stress precedes the occurrence of hypertension in genetic hypertensive animals^{39,40,41}. The source of oxidative stress could be renal in origin. However, it is possible that primary systemic oxidative stress caused by elevated and activated circulating leukocytes, capable of releasing reactive oxygen species (ROS), can be directly delivered into kidney by these mobile blood-borne cells. In animal studies, Shen et al.⁴² found a highly significant elevation in the total leukocyte, neutrophil and monocyte counts, as well as in the level of activated neutrophils and monocytes in Dahl salt-sensitive rat, a salt-sensitive hypertensive model, but not in Dahl salt-resistance rats. Interestingly, in both spontaneously hypertensive rats (SHR)⁴³ and Sabra rats⁴⁴, another model of salt-sensitive hypertension, elevation and activation of leukocytes precedes the occurrence of experimental hypertension together with increased superoxide ($O_2^{\cdot-}$) release from polymorphonuclear leukocytes (PMNLs)⁴⁴.

Hypertensive patients have been reported to exhibit a decreased activity of cytosolic and mitochondrial superoxide dismutase in PMNLs and increased rates of lipid peroxidation⁴⁵. It has also been observed that $O_2^{\cdot-}$ and hydrogen peroxide (H_2O_2) production by PMNLs^{46,47,48} and levels of lipid peroxides⁴⁹ were higher in patients with uncontrolled hypertension compared to controls. Most importantly, Kristal et al.⁵⁰ reported that in patients with essential hypertension, the peripheral PMNLs are in a primed state relative to healthy subjects and upon stimulation, release $O_2^{\cdot-}$ at a faster rate. Activated neutrophils may suppress nitric oxide synthesis (iNOS) expression⁵¹, which could profoundly deteriorate kidney function⁵².

Once the innate immune system has been activated and renal damage initiated, it is possible that low-grade inflammation in tubulointerstitial areas of the kidney could be maintained by autoimmune reactivity⁵³. In this case, circulating lymphocytes could experience an autoantibody attack⁵⁴. In the late 1970s, Takeichi et al.^{55,56} found that the SHR not only has fewer circulating T-lymphocytes but also a depressed functionality in this lymphocyte population. They later reported that a circulating natural thymocytotoxic autoantibody was present in the SHR and this “naturally occurring autoantibody” was specific for the T-lymphocytes⁵⁴. Therefore, even though we didn’t measure autoantibody due to the limitation of this cohort study, a similar or the same autoantibody found in above hypertensive animal model exists in human is possible. This auto-attack could reduce their T cell levels as we saw in the present study and at the same time release ROS and Ang II⁵⁷. Concurrently, T lymphocytes could infiltrate into the kidney including the tubulointerstitial area⁵⁸ and directly release ROS and Ang II. A recent study in Dahl SS rats showed that T lymphocytes that infiltrate into the kidney possess components of the renin-angiotensin system and can increase Ang II levels^{59,60}. Furthermore, activated lymphocytes increase tissue-homing receptors CD44 and CCR5 and could further attract more lymphocytes into tissue⁶¹, and further reduce the circulating lymphocyte levels. Hence, increased intrarenal ROS, Ang II and inflammation by renal infiltration of T lymphocytes could have enhanced renal arteriolar damage and nephrosclerosis. Therefore, blood pressure increase and /or hypertension could result²⁴.

If the above hypothesis is true, changes in levels of circulating specific leukocyte types should precede and contribute to the renal damage and dysfunction. Indeed, our current

study has shown that increased neutrophil levels and decreased lymphocyte levels are associated with the increased incidence of CKD in the whole ARIC population, suggesting that specific leukocyte-related renal mechanism of essential HT is possible.

Furthermore, as we expected, our data showed that African-Americans tended to have similar but stronger patterns of association than whites between above circulating specific leukocyte types and CKD incidence in the ARIC study population, although the differences between race groups were not statistically significant. These results suggest that kidney dysfunction in African-Americans is more sensitive to the level of circulating specific leukocyte types than in whites, indicating that those leukocytes may be significantly involved in the kidney damage or hypertensive nephrosclerosis in African-Americans due to their genetic characteristics^{62,63}.

The next logical question, then, is: 1) what is the mechanism of those specific circulating leukocyte-related renal damage, and 2) why is kidney dysfunction in African-Americans more sensitive to the level of specific leukocyte types?

Glomerular hyperfiltration in human has been hypothesized to reflect increased glomerular capillary pressure, which is a primary mediator of glomerular capillary damage in rat models^{64,65}. Increased GFR has been connected to glomerular structural changes and renal dysfunction^{66,67}, particularly in elderly, obese, diabetic or black subjects⁶⁸. Studies have shown that GFR is significantly higher in blacks than in whites in hypertensive patients with high salt intake^{69,70} suggesting that increased susceptibility to glomerular hyperfiltration of African-Americans may explain their proclivity to progressive renal injury associated with essential HT. However, most of previous studies on glomerular hyperfiltration had only a short duration and small samples, and whether glomerular hyperfiltration or glomerular HT precedes and initiates progressive renal damage and CKD in normal population is unknown. Our results in ARIC population have shown that at each visit, African-Americans have higher GFR than whites. To our knowledge, this is the first study with a longitudinal design and large population sample to show racial differences in GFR in normal population, indicating a mechanism---impaired GFR autoregulation and glomerular hyperfiltration---that may contribute to the racial disparity in CKD and HT.

The last question is: what is the relationship between inflammation and glomerular hyperfiltration? Studies in animals have shown that prevention of renal injury by suppression of the inflammatory process in rats with subtotal renal ablation could partially due to decrease in glomerular HT and hyperfiltration in remnant nephrons. Anti-inflammatory drugs mycophenolate mofetil (MMF)¹¹ and polysulphate pentosan (PPS)¹² have been found to significantly preserve afferent arteriolar function and reduce single-nephron GFR and reduce glomerular sclerosis, tubulointerstitial damage, lymphocyte and macrophage infiltration. Also, increased glomerular pressure is associated with arteriolar lesions and tubulointerstitial inflammation⁷¹. In our current study, we believe that leukocyte associated arteriolopathy of afferent arteriolar contributes to glomerular hyperfiltration leading to the progressive renal disease.

Our study has a number of limitations. First, although we controlled for some major cardiovascular risk factors, the existence of unrecognized confounding variables is always possible. However, this is likely to be small. Second, since urinalysis was not available in the ARIC study, patients who had CKD but normal eGFR (eGFR > 60 mL/min/1.73 m²) at baseline could still be included in the study sample; detection of proteinuria on urinalysis would have allowed us to exclude them. Finally, the finding at each visit of higher mean eGFR and BP in African-Americans than in whites seems inconsistent with animal findings. In animal studies, higher BP is always accompanied by a lower level of eGFR once kidney

function deteriorates to a certain level. It is possible that the observation time in our study was not long enough to see a decline in eGFR in African-Americans.

In summary, this study presents evidence that increased levels of circulating neutrophils or decreased levels of circulating lymphocytes play an important independent role in the progression of kidney disease. Our results suggest that activated circulating leukocytes may be involved in renal arteriolar dysfunction, which could lead to glomerular hyperfiltration and CKD. These findings lend indirect support to a circulating leukocyte-related renal mechanism of essential HT, especially in African-Americans, who may have a genetic susceptibility to renal damage from neutrophils and lymphocytes,

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Reference List

1. Elsayed EF, Tighiouart H, Griffith J, Kurth T, Levey AS, Salem D, Sarnak MJ, Weiner DE. Cardiovascular disease and subsequent kidney disease. *Arch.Intern.Med.* 2007; 167:1130–1136. [PubMed: 17563020]
2. Bang H, Vupputuri S, Shoham DA, Klemmer PJ, Falk RJ, Mazumdar M, Gipson D, Colindres RE, Kshirsagar AV. SCReening for Occult RENal Disease (SCORED): a simple prediction model for chronic kidney disease. *Arch.Intern.Med.* 2007; 167:374–381. [PubMed: 17325299]
3. Knight EL, Rimm EB, Pai JK, Rexrode KM, Cannuscio CC, Manson JE, Stampfer MJ, Curhan GC. Kidney dysfunction, inflammation, and coronary events: a prospective study. *J Am Soc Nephrol.* 2004; 15:1897–1903. [PubMed: 15213279]
4. Shlipak MG, Fried LF, Crump C, Bleyer AJ, Manolio TA, Tracy RP, Furberg CD, Psaty BM. Elevations of inflammatory and procoagulant biomarkers in elderly persons with renal insufficiency. *Circulation.* 2003; 107:87–92. [PubMed: 12515748]
5. Kshirsagar AV, Bombardier AS, Bang H, Gerber LM, Vupputuri S, Shoham DA, Mazumdar M, Ballantyne CM, Paparello JJ, Klemmer PJ. Association of C-reactive protein and microalbuminuria (from the National Health and Nutrition Examination Surveys, 1999 to 2004). *Am J Cardiol.* 2008; 101:401–406. [PubMed: 18237609]
6. Tonelli M, Sacks F, Pfeffer M, Jhangri GS, Curhan G. Biomarkers of inflammation and progression of chronic kidney disease. *Kidney Int.* 2005; 68:237–245. [PubMed: 15954913]
7. Bash LD, Erlinger TP, Coresh J, Marsh-Manzi J, Folsom AR, Astor BC. Inflammation, hemostasis, and the risk of kidney function decline in the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Kidney Dis.* 2009; 53:596–605. [PubMed: 19110358]
8. Erlinger TP, Tarver-Carr ME, Powe NR, Appel LJ, Coresh J, Eberhardt MS, Brancati FL. Leukocytosis, hypoalbuminemia, and the risk for chronic kidney disease in US adults. *Am J Kidney Dis.* 2003; 42:256–263. [PubMed: 12900806]
9. Fried L, Solomon C, Shlipak M, Seliger S, Stehman-Breen C, Bleyer AJ, Chaves P, Furberg C, Kuller L, Newman A. Inflammatory and prothrombotic markers and the progression of renal disease in elderly individuals. *J Am Soc Nephrol.* 2004; 15:3184–3191. [PubMed: 15579522]
10. Rees AJ. The role of infiltrating leukocytes in progressive renal disease: implications for therapy. *Nat.Clin.Pract.Nephrol.* 2006; 2:348–349. [PubMed: 16932458]
11. Tapia E, Franco M, Sanchez-Lozada LG, Soto V, Avila-Casado C, Santamaria J, Quiroz Y, Rodriguez-Iturbe B, Herrera-Acosta J. Mycophenolate mofetil prevents arteriopathy and renal injury in subtotal ablation despite persistent hypertension. *Kidney Int.* 2003; 63:994–1002. [PubMed: 12631080]
12. Bobadilla NA, Tack I, Tapia E, Sanchez-Lozada LG, Santamaria J, Jimenez F, Striker LJ, Striker GE, Herrera-Acosta J. Pentosan polysulfate prevents glomerular hypertension and structural injury

- despite persisting hypertension in 5/6 nephrectomy rats. *J.Am.Soc.Nephrol.* 2001; 12:2080–2087. [PubMed: 11562406]
13. Wolfe RA, Port FK, Webb RL, Bloembergen WE, Hirth R, Young EW, Ojo AO, Strawderman RL, Parekh R, Stack A, Tedeschi PJ, Hulbert-Shearon T, Ashby VB, Callard S, Hanson J, Jain A, Meyers-Purkiss A, Roys E, Brown P, Wheeler JR, Jones CA, Greer JW, Agodoa LY. Introduction to the excerpts from the United States Renal Data System 1999 Annual Data Report. *Am J Kidney Dis.* 1999; 34:S1–S3. [PubMed: 10430996]
 14. Brancati FL, Whelton PK, Whittle JC, Klag MJ. Epidemiologic analysis of existing data to investigate hypertensive renal disease: an example from the Maryland End-Stage Renal Disease Registry. *Am J Kidney Dis.* 1993; 21:15–24. [PubMed: 8465831]
 15. Feldman HI, Klag MJ, Chiapella AP, Whelton PK. End-stage renal disease in US minority groups. *Am J Kidney Dis.* 1992; 19:397–410. [PubMed: 1585925]
 16. Ferguson R, Grim CE, Opgenorth TJ. The epidemiology of end-state renal disease: the six-year South-Central Los Angeles experience, 1980–85. *Am J Public Health.* 1987; 77:864–865. [PubMed: 3592045]
 17. The ARIC investigators: The Atherosclerosis Risk in Communities(ARIC) Study:design and objectives. *Am J Epidemiol.* 1989; 129:687–702. [PubMed: 2646917]
 18. Reich D, Nalls MA, Kao WH, Akylbekova EL, Tandon A, Patterson N, Mullikin J, Hsueh WC, Cheng CY, Coresh J, Boerwinkle E, Li M, Waliszewska A, Neubauer J, Li R, Leak TS, Ekunwe L, Files JC, Hardy CL, Zmuda JM, Taylor HA, Ziv E, Harris TB, Wilson JG. Reduced neutrophil count in people of African descent is due to a regulatory variant in the Duffy antigen receptor for chemokines gene. *PLoS.Genet.* 2009; 5:e1000360. [PubMed: 19180233]
 19. Freedman DS, Gates L, Flanders WD, Van Assendelft OW, Barboriak JJ, Joesoef MR, Byers T. Black/white differences in leukocyte subpopulations in men. *Int.J.Epidemiol.* 1997; 26:757–764. [PubMed: 9279607]
 20. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann.Intern.Med.* 1999; 130:461–470. [PubMed: 10075613]
 21. Shoham DA, Vupputuri S, Diez Roux AV, Kaufman JS, Coresh J, Kshirsagar AV, Zeng D, Heiss G. Kidney disease in life-course socioeconomic context: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Kidney Dis.* 2007; 49:217–226. [PubMed: 17261424]
 22. Tobin MD, Sheehan NA, Scurrah KJ, Burton PR. Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. *Stat.Med.* 2005; 24:2911–2935. [PubMed: 16152135]
 23. Wu J, Kraja AT, Oberman A, Lewis CE, Ellison RC, Arnett DK, Heiss G, Lalouel JM, Turner ST, Hunt SC, Province MA, Rao DC. A summary of the effects of antihypertensive medications on measured blood pressure. *Am.J.Hypertens.* 2005; 18:935–942. [PubMed: 16053990]
 24. Tian N, Penman AD, Mawson AR, Manning RD Jr, Flessner MF. Association between circulating specific leukocyte types and blood pressure: the atherosclerosis risk in communities (ARIC) study. *J.Am.Soc.Hypertens.* 2010; 4:272–283. [PubMed: 20980213]
 25. Tian N, Thrasher KD, Gundy PD, Hughson MD, Manning RD Jr. Antioxidant treatment prevents renal damage and dysfunction and reduces arterial pressure in salt-sensitive hypertension. *Hypertension.* 2005; 45:934–939. [PubMed: 15837840]
 26. Tian N, Rose RA, Jordan S, Dwyer TM, Hughson MD, Manning RD Jr. N-Acetylcysteine improves renal dysfunction, ameliorates kidney damage and decreases blood pressure in salt-sensitive hypertension. *J.Hypertens.* 2006; 24:2263–2270. [PubMed: 17053549]
 27. Tian N, Moore RS, Phillips WE, Lin L, Braddy S, Pryor JS, Stockstill RL, Hughson MD, Manning RD Jr. NADPH oxidase contributes to renal damage and dysfunction in Dahl salt-sensitive hypertension. *Am J Physiol Regul.Integr.Comp Physiol.* 2008; 295:R1858–R1865. [PubMed: 18922960]
 28. Tian N, Moore RS, Braddy S, Rose RA, Gu JW, Hughson MD, Manning RD Jr. Interactions between oxidative stress and inflammation in salt-sensitive hypertension. *Am.J.Physiol Heart Circ.Physiol.* 2007; 293:H3388–H3395. [PubMed: 17921322]

29. Tian N, Gu JW, Jordan S, Rose RA, Hughson MD, Manning RD Jr. Immune suppression prevents renal damage and dysfunction and reduces arterial pressure in salt-sensitive hypertension. *Am J Physiol Heart Circ.Physiol.* 2007; 292:H1018–H1025. [PubMed: 17040973]
30. Kobori H, Ozawa Y, Suzaki Y, Nishiyama A. Enhanced intrarenal angiotensinogen contributes to early renal injury in spontaneously hypertensive rats. *J Am Soc Nephrol.* 2005; 16:2073–2080. [PubMed: 15888567]
31. Kobori H, Nishiyama A. Effects of tempol on renal angiotensinogen production in Dahl salt-sensitive rats. *Biochem.Biophys.Res.Comm.* 2004; 315:746–750. [PubMed: 14975764]
32. Vaziri ND, Rodriguez-Iturbe B. Mechanisms of disease: oxidative stress and inflammation in the pathogenesis of hypertension. *Nat.Clin.Pract.Nephrol.* 2006; 2:582–593. [PubMed: 17003837]
33. Wilcox CS. Oxidative stress and nitric oxide deficiency in the kidney: a critical link to hypertension? *Am.J Physiol Regul.Integr.Comp Physiol.* 2005; 289:R913–R935. [PubMed: 16183628]
34. Rubanyi GM, Vanhoutte PM. Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am J Physiol.* 1986; 250:H822–H827. [PubMed: 3010744]
35. Lahera V, Salom MG, Miranda-Guardiola F, Moncada S, Romero JC. Effects of NG-nitro-L-arginine methyl ester on renal function and blood pressure. *Am J Physiol.* 1991; 261:F1033–F1037. [PubMed: 1750517]
36. Manning RD Jr, Meng S, Tian N. Renal and vascular oxidative stress and salt-sensitivity of arterial pressure. *Acta Physiol Scand.* 2003; 179:243–250. [PubMed: 14616240]
37. Rodriguez-Iturbe B, Vaziri ND, Herrera-Acosta J, Johnson RJ. Oxidative stress, renal infiltration of immune cells, and salt-sensitive hypertension: all for one and one for all. *Am J Physiol Renal Physiol.* 2004; 286:F606–F616. [PubMed: 15001451]
38. Meneton P, Jeunemaitre X, de Wardener HE, MacGregor GA. Links between dietary salt intake, renal salt handling, blood pressure, and cardiovascular diseases. *Physiol Rev.* 2005; 85:679–715. [PubMed: 15788708]
39. Chabrashvili T, Tojo A, Onozato ML, Kitiyakara C, Quinn MT, Fujita T, Welch WJ, Wilcox CS. Expression and cellular localization of classic NADPH oxidase subunits in the spontaneously hypertensive rat kidney. *Hypertension.* 2002; 39:269–274. [PubMed: 11847196]
40. Meng S, Roberts LJ, Cason GW, Curry TS, Manning RD Jr. Superoxide dismutase and oxidative stress in Dahl salt-sensitive and -resistant rats. *Am.J.Physiol Regul.Integr.Comp Physiol.* 2002; 283:R732–R738. [PubMed: 12185008]
41. Meng S, Cason GW, Gannon AW, Racusen LC, Manning RD Jr. Oxidative stress in Dahl salt-sensitive hypertension. *Hypertension.* 2003; 41:1346–1352. [PubMed: 12719439]
42. Shen K, DeLano FA, Zweifach BW, Schmid-Schonbein GW. Circulating leukocyte counts, activation, and degranulation in Dahl hypertensive rats. *Circ.Res.* 1995; 76:276–283. [PubMed: 7834839]
43. Schmid-Schonbein GW, Seiffge D, DeLano FA, Shen K, Zweifach BW. Leukocyte counts and activation in spontaneously hypertensive and normotensive rats. *Hypertension.* 1991; 17:323–330. [PubMed: 1999363]
44. Sela S, Mazor R, Amsalam M, Yagil C, Yagil Y, Kristal B. Primed polymorphonuclear leukocytes, oxidative stress, and inflammation antecede hypertension in the Sabra rat. *Hypertension.* 2004; 44:764–769. [PubMed: 15452031]
45. Rahman I, Nath N. Glutathione & its redox system, superoxide anion & superoxide dismutases of polymorphonuclear leukocytes in essential hypertension. *Indian J Med.Res.* 1988; 88:64–70. [PubMed: 2846441]
46. Sagar S, Kallo IJ, Kaul N, Ganguly NK, Sharma BK. Oxygen free radicals in essential hypertension. *Mol.Cell Biochem.* 1992; 111:103–108. [PubMed: 1588931]
47. Kumar KV, Das UN. Are free radicals involved in the pathobiology of human essential hypertension? *Free Radic.Res.Comm.* 1993; 19:59–66. [PubMed: 8225035]
48. Pontremoli S, Salamino F, Sparatore B, De TR, Patrone M, Tizianello A, Melloni E. Enhanced activation of the respiratory burst oxidase in neutrophils from hypertensive patients. *Biochem.Biophys.Res.Comm.* 1989; 158:966–972. [PubMed: 2537641]

49. Prabha PS, Das UN, Koratkar R, Sagar PS, Ramesh G. Free radical generation, lipid peroxidation and essential fatty acids in uncontrolled essential hypertension. *Prostaglandins Leukot.Essent.Fatty Acids*. 1990; 41:27–33. [PubMed: 2174565]
50. Kristal B, Shurtz-Swirski R, Chezar J, Manaster J, Levy R, Shapiro G, Weissman I, Shasha SM, Sela S. Participation of peripheral polymorphonuclear leukocytes in the oxidative stress and inflammation in patients with essential hypertension. *Am J Hypertens*. 1998; 11:921–928. [PubMed: 9715783]
51. Morton J, Coles B, Wright K, Gallimore A, Morrow JD, Terry ES, Anning PB, Morgan BP, Dioszeghy V, Kuhn H, Chaitidis P, Hobbs AJ, Jones SA, O'Donnell VB. Circulating neutrophils maintain physiological blood pressure by suppressing bacteria and IFN γ -dependent iNOS expression in the vasculature of healthy mice. *Blood*. 2008; 111:5187–5194. [PubMed: 18281503]
52. Tian N, Gannon AW, Khalil RA, Manning RD Jr. Mechanisms of salt-sensitive hypertension: role of renal medullary inducible nitric oxide synthase. *Am.J.Physiol Regul.Integr.Comp Physiol*. 2003; 284:R372–R379. [PubMed: 12399250]
53. Caetano EP, Zatz R, Praxedes JN. The clinical diagnosis of hypertensive nephrosclerosis--how reliable is it? *Nephrol Dial.Transplant*. 1999; 14:288–290. [PubMed: 10069175]
54. Takeichi N, Ba D, Kobayashi H. Natural cytotoxic autoantibody against thymocytes in spontaneously hypertensive rats. *Cell Immunol*. 1981; 60:181–190. [PubMed: 6972263]
55. Takeichi N, Boone CW. Spontaneous rosette formation of rat thymus cells with guinea pig erythrocytes. *Cell Immunol*. 1976; 27:52–59. [PubMed: 1086724]
56. Takeichi N, Suzuki K, Okayasu T, Kobayashi H. Immunological depression in spontaneously hypertensive rats. *Clin.Exp.Immunol*. 1980; 40:120–126. [PubMed: 6993074]
57. Hoch NE, Guzik TJ, Chen W, Deans T, Maalouf SA, Gratz P, Weyand C, Harrison DG. Regulation of T-cell function by endogenously produced angiotensin II. *Am J Physiol Regul.Integr.Comp Physiol*. 2009; 296:R208–R216. [PubMed: 19073907]
58. Rodriguez-Iturbe B, Quiroz Y, Herrera-Acosta J, Johnson RJ, Pons HA. The role of immune cells infiltrating the kidney in the pathogenesis of salt-sensitive hypertension. *J Hypertens*. 2002 Suppl 20:S9–S14.
59. De MC, Das S, Lund H, Mattson DL. T lymphocytes mediate hypertension and kidney damage in Dahl salt-sensitive rats. *Am.J.Physiol Regul.Integr.Comp Physiol*. 2010; 298:R1136–R1142. [PubMed: 20147611]
60. Miguel CD, Guo C, Lund H, Feng D, Mattson DL. Infiltrating T lymphocytes in the kidney increase oxidative stress and participate in the development of hypertension and renal disease. *Am.J.Physiol Renal Physiol*. 2011; 300:F734–F742. [PubMed: 21159736]
61. Guzik TJ, Hoch NE, Brown KA, McCann LA, Rahman A, Dikalov S, Goronzy J, Weyand C, Harrison DG. Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. *J Exp.Med*. 2007; 204:2449–2460. [PubMed: 17875676]
62. Kopp JB, Smith MW, Nelson GW, Johnson RC, Freedman BI, Bowden DW, Oleksyk T, McKenzie LM, Kajiyama H, Ahuja TS, Berns JS, Briggs W, Cho ME, Dart RA, Kimmel PL, Korbet SM, Michel DM, Mokrzycki MH, Schelling JR, Simon E, Trachtman H, Vlahov D, Winkler CA. MYH9 is a major-effect risk gene for focal segmental glomerulosclerosis. *Nat.Genet*. 2008; 40:1175–1184. [PubMed: 18794856]
63. Kao WH, Klag MJ, Meoni LA, Reich D, Berthier-Schaad Y, Li M, Coresh J, Patterson N, Tandon A, Powe NR, Fink NE, Sadler JH, Weir MR, Abboud HE, Adler SG, Divers J, Iyengar SK, Freedman BI, Kimmel PL, Knowler WC, Kohn OF, Kramp K, Leehey DJ, Nicholas SB, Pahl MV, Schelling JR, Sedor JR, Thornley-Brown D, Winkler CA, Smith MW, Parekh RS. MYH9 is associated with nondiabetic end-stage renal disease in African Americans. *Nat.Genet*. 2008; 40:1185–1192. [PubMed: 18794854]
64. Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. *Am.J.Physiol*. 1981; 241:F85–F93. [PubMed: 7246778]
65. Hostetter TH, Rennke HG, Brenner BM. Compensatory renal hemodynamic injury: a final common pathway of residual nephron destruction. *Am.J.Kidney Dis*. 1982; 1:310–314. [PubMed: 7072714]

66. Henegar JR, Bigler SA, Henegar LK, Tyagi SC, Hall JE. Functional and structural changes in the kidney in the early stages of obesity. *J.Am.Soc.Nephrol.* 2001; 12:1211–1217. [PubMed: 11373344]
67. Schmieder RE, Messerli FH, Garavaglia G, Nunez B. Glomerular hyperfiltration indicates early target organ damage in essential hypertension. *JAMA.* 1990; 264:2775–2780. [PubMed: 2146412]
68. Boero R, Pignataro A, Quarello F. Salt intake and kidney disease. *J.Nephrol.* 2002; 15:225–229. [PubMed: 12113591]
69. Parmer RJ, Stone RA, Cervenka JH. Renal hemodynamics in essential hypertension. Racial differences in response to changes in dietary sodium. *Hypertension.* 1994; 24:752–757. [PubMed: 7995633]
70. Kotchen TA, Piering AW, Cowley AW, Grim CE, Gaudet D, Hamet P, Kaldunski ML, Kotchen JM, Roman RJ. Glomerular hyperfiltration in hypertensive African Americans. *Hypertension.* 2000; 35:822–826. [PubMed: 10720601]
71. Sanchez-Lozada LG, Tapia E, Johnson RJ, Rodriguez-Iturbe B, Herrera-Acosta J. Glomerular hemodynamic changes associated with arteriolar lesions and tubulointerstitial inflammation. *Kidney Int.* 2003 Suppl:S9–S14.

Fig. 1. Comparison of adjusted Hazard Ratio for incident chronic kidney disease (CKD) by tertile (T) of neutrophil level in African-Americans and whites (adjusted for age, sex, diabetes, BMI, smoking, drinking, and total cholesterol).

Fig. 2.
Comparison of adjusted Hazard Ratio for incident chronic kidney disease (CKD) by tertile (T) of lymphocyte level African-Americans and whites (adjusted for age, sex, diabetes, BMI, smoking, drinking, and total cholesterol).

Fig. 3.

Vector plots of mean systolic blood pressure (SBP) vs mean estimated glomerular filtration rate (eGFR) at each visit, by race (values for visit 3 obtained by interpolation between visits 2 and 4; adjusted for age, sex, diabetes, BMI, smoking, drinking, and total cholesterol).

Table 1

Baseline clinical characteristics of study population, by tertile of neutrophil count.

Characteristic	Whites			P for trend
	Tertile 1 (highest) 65%–88% [N=2,159]	Tertile 2 58%–64% [N=2,224]	Tertile 3 (lowest) 6%–57% [N=2,293]	
Neutrophils, %	69.7	61.0	71.7	--
Age, yrs	53.7 (0.1)	54.0 (0.1)	54.1 (0.1)	0.01
Men, %	46.1	43.9	39.2	<.0001
Systolic BP, mmHg	118.5 (0.4)	117.8 (0.4)	116.7 (0.3)	0.0003
Diastolic BP, mmHg	71.5 (0.2)	71.2 (0.2)	71.2 (0.2)	<.0001
Diabetes, %	8.0	6.3	5.2	0.0002
Current smoking, %	34.5	24.4	20.7	<.0001
Current drinking, %	71.2	67.9	70.8	0.80
BMI, kg/m ²	26.1 (0.1)	26.4 (0.1)	26.1 (0.1)	0.85
Total cholesterol, mmol/L	5.4 (0.02)	5.5 (0.02)	5.6 (0.02)	<.0001
	African-Americans			
	Tertile 1 (highest) 54%–84% [N=1,114]	Tertile 2 43%–53% [N=1,112]	Tertile 3 (lowest) 6%–42% [N=1,154]	
Neutrophils, %	60.9	48.1	33.4	--
Age, yrs	53.3 (0.2)	53.4 (0.2)	53.1 (0.2)	0.44
Men, %	40.8	36.2	34.6	0.002
Systolic BP, mmHg	129.9 (0.6)	127.3 (0.6)	127.1 (0.6)	0.001
Diastolic BP, mmHg	78.8 (0.4)	79.2 (0.4)	80.1 (0.4)	0.01
Diabetes, %	19.3	17.5	15.3	0.01
Current smoking, %	33.3	31.1	26.3	0.0002
Current drinking, %	36.1	33.9	28.5	0.0001
BMI, kg/m ²	28.9 (0.2)	29.6 (0.2)	29.3 (0.2)	0.06
Total cholesterol, mmol/L	5.5 (0.03)	5.5 (0.03)	5.6 (0.03)	0.003

Abbreviations: BP, blood pressure; BMI, body mass index. Values are means (SE) or percentages