Markers of inflammation predict the long-term risk of developing chronic kidney disease: a population-based cohort study

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In animal models, inflammatory processes have been shown to have an important role in the development of kidney disease. In humans, however, the independent relation between markers of inflammation and the risk of chronic kidney disease (CKD) is not known. To clarify this, we examined the relationship of several inflammatory biomarker levels (high-sensitivity C-reactive protein, tumor necrosis factor-α receptor 2, white blood cell count, and interleukin-6) with the risk of developing CKD in a population-based cohort of up to 4926 patients with 15 years of follow-up. In cross-sectional analyses, we found that all these inflammation markers were positively associated with the outcome of interest, prevalent CKD. However, in longitudinal analyses examining the risk of developing incident CKD among those who were CKD-free at baseline, only tumor necrosis factor-α receptor 2, white blood cell count, and interleukin-6 levels (hazard ratios comparing highest with the lowest tertile of 2.10, 1.90, and 1.45, respectively), and not C-reactive protein (hazard ratio 1.09), were positively associated with incident CKD. Thus, elevations of most markers of inflammation predict the risk of developing CKD. Each marker should be independently verified.

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KEYWORDS: chronic kidney disease; CRP; inflammation; tumor necrosis factor-alpha

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Chronic kidney disease (CKD) involves several pathophysiological mechanisms that are analogous to atherosclerosis.[1] Inflammatory processes are considered to have a key role in atherosclerosis development.[2] Markers of inflammation are implicated in the development of diabetes mellitus[3,4] and hypertension,[5] which are strong risk factors for CKD.[6] In animal models, inflammatory processes have been shown to have an important role in kidney disease development.[7–11] However, in humans, the independent relationship between markers of inflammation and the risk of developing CKD is not clear. In this context, we examined the independent relationship between markers of inflammation and the risk of developing CKD over a period of 15 years in a population-based cohort study of predominantly White subjects from Wisconsin who were free of CKD at baseline.

RESULTS

For the cross-sectional analysis, after exclusions (Figure 1), 4124 subjects with complete covariate data were available for the white blood cell (WBC) count and C-reactive protein (CRP) analyses, and 1503 subjects for the tumor necrosis factor-alpha receptor 2 level (TNF-αR2) and interleukin (IL)-6 analyses. Similarly, for the longitudinal analysis (Figure 2), 2877 subjects were available for the WBC count and CRP analyses, and 1508 subjects for the TNF-αR2 and IL-6 analyses.

Table 1 presents the baseline characteristics of the cohort for the serum TNF-αR2-incident CKD analysis. Subjects with higher TNF-αR2 levels were more likely to be older, a current smoker, a never or former drinker, more likely to have <high school education, a higher body mass index, diabetes mellitus, hypertension, higher systolic blood pressure, less likely to be a former smoker, and less likely to have >high school education.

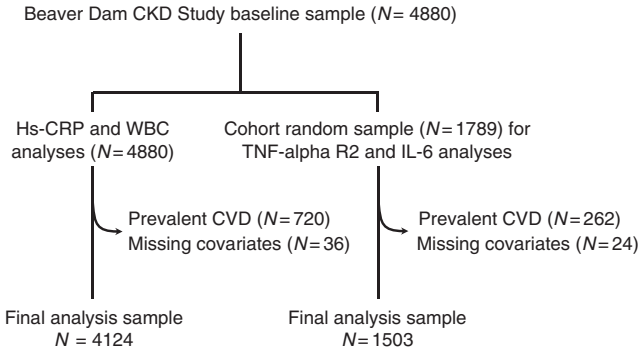

Figure 1 | Flowchart for sample construction of the cross-sectional analysis. CKD, chronic kidney disease; CVD, cardiovascular disease; Hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; TNF, tumor necrosis factor; WBC, white blood cell.

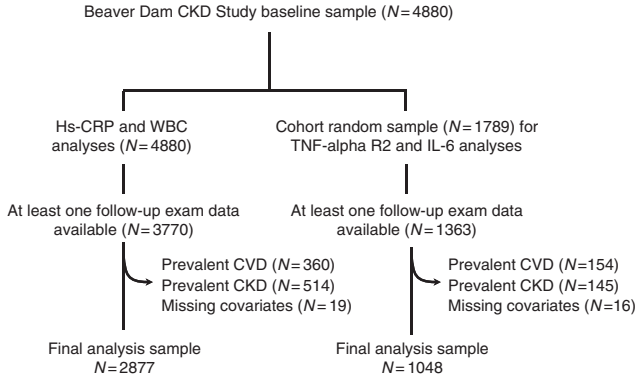

Figure 2 | Flowchart for sample construction of the longitudinal analysis. CKD, chronic kidney disease; CVD, cardiovascular disease; Hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; TNF, tumor necrosis factor; WBC, white blood cell.

Similar to Table 1, we examined the baseline characteristics of the cohort by increasing tertiles of other markers of inflammation, including serum CRP, IL-6, and WBC count (data not presented). We found that the findings were essentially similar to the results for TNF-α receptor 2 analysis in Table 1, except that the inverse association with former smoking was not statistically significant for IL-6 ($P = 0.071$).

Table 2 presents the distribution of estimated glomerular filtration rate (eGFR) categories in the baseline cohort and how the mean level of each marker of inflammation varied with increasing severity of kidney function. Approximately 85% of the study subjects had an eGFR $\geqslant$ 60 ml/min per 1.73 m^2, whereas 0.5% had an eGFR $<$ 30 ml/min per 1.73 m^2. We found that the mean level of each marker of inflammation increased with worsening kidney function.

Table 3 presents the cross-sectional association between increasing categories of serum CRP, TNF-αR2, IL-6, WBC count, and prevalent CKD. Overall, all the markers of inflammation were found to be positively associated with prevalent CKD in both the age- and sex-adjusted model and the multivariable-adjusted model. Corresponding models of trend were also statistically significant.

Table 4 presents the longitudinal associations between increasing categories of serum CRP, TNF-αR2, IL-6, WBC count, and 15-year incident CKD. We ran multivariable models initially without (multivariable-adjusted hazard ratio 1) and subsequently with adjustment for diabetes and hypertension (multivariable-adjusted hazard ratio 2) to study the effect of statistical adjustment for these variables, which are potential mediators. We found that there was no appreciable attenuation of the association between these markers of inflammation and 15-year incident CKD with additional adjustment for diabetes and hypertension.

In Table 4, for the CRP analysis, there was no association between increasing CRP levels and incident CKD (P-trend = 0.42 in the multivariable model 2). In contrast, for the TNF-αR2 analysis, a positive association between increasing TNF-αR2 levels and incident CKD (P-trend $<$ 0.0001 in the multivariable mode 2) was present. Similar positive associations, although of lesser magnitude, were observed for WBC count and IL-6.

We also performed several supplementary analyses. First, to examine whether the positive association observed between increasing TNF-αR2 levels and 15-year incident CKD in the longitudinal analysis was independent of other inflammatory markers (CRP, WBC count, and IL-6 levels), we additionally adjusted for levels of these markers as a continuous variable in the multivariable model. The results were attenuated, but a positive association persisted for TNF-αR2. Compared with the lowest tertile of TNF-αR2 (referent), the HR (95% confidence interval (CI)) of CKD was 1.24 (0.90, 1.71) in the second tertile and 1.68 (1.29, 2.19) in the highest tertile; P-trend = 0.002. Second, to avoid the possibility of reverse causality, we repeated the main analysis after excluding the CKD cases occurring during the initial 5 years of follow-up. The overall results were similar; however, the magnitude of association was slightly attenuated for TNF-αR2 results. For the CRP analysis, compared with the lowest tertile (referent), the HR (95% CI) of CKD was 1.06 (0.81, 1.39) in the second tertile and 1.09 (0.86, 1.38) in the highest tertile; P-trend = 0.42. For TNF-αR2 analysis, compared with the lowest tertile (referent), the HR (95% CI) of CKD was 1.47 (0.89, 2.43) in the second tertile and 1.90 (1.11, 3.25) in the highest tertile; P-trend $<$ 0.0001.

Finally, defining CKD as both an eGFR falling below 60 ml/min per 1.73 m^2 and a decrease in eGFR of at least 25% over the follow-up period as suggested by Bash *et al.*,[12] there were 350 incident cases of CKD for the CRP analysis and 143 incident CKD cases for the TNF-αR2 analysis over the 15-year follow-up period. For the CRP analysis, compared with the lowest tertile (referent), the HR (95% CI) of CKD was 1.12 (0.84, 1.49) in the second tertile and 1.08 (0.88, 1.33) in the highest tertile; P-trend = 0.51. For TNF-αR2 analysis, compared with the lowest tertile (referent), the HR (95% CI) of CKD was 1.54 (0.88, 2.70) in the second tertile and 2.04 (1.09, 3.82) in the highest tertile; P-trend $<$ 0.0001.

Table 1 | Baseline characteristics of cohort for the TNF-α receptor 2 analysis

Characteristic[a]	Total	Tertile 1	Tertile 2	Tertile 3[b]	*P*-value[c]
Age, years	58.4 (0.3)	54.8 (0.5)	57.8 (0.5)	62.5 (0.5)	<0.0001
Females, %	55.3	53.0	55.7	57.3	0.2536
Education, %					
Below high school	19.6	13.2	16.0	29.5	<0.0001
High school	47.1	45.3	52.9	43.3	0.5956
Above high school	33.3	41.6	31.1	27.2	<0.0001
History of smoking, %					
Never	44.0	43.3	43.7	45.0	0.6473
Former	34.8	38.4	34.9	31.2	0.0473
Current	21.2	18.3	21.4	23.8	0.0488
History of alcohol intake categories, %					
Never	3.0	2.3	1.7	4.9	0.0480
Former	7.1	4.9	6.3	10.0	0.0086
Current drinker, <3 drinks per day	81.0	82.8	81.4	78.8	0.1771
Current drinker, ⩾3 drinks per day	9.0	10.0	10.6	6.3	0.0862
Body mass index (BMI), kg/m^2	28.7 (0.2)	27.5 (0.3)	29.1 (0.3)	29.7 (0.3)	<0.0001
Diabetes mellitus, %	8.7	6.0	8.0	12.0	0.0052
Glycosylated hemoglobin, %	6.0 (1.5)	5.8 (0.1)	5.9 (0.1)	6.2 (0.1)	0.0025
Serum total cholesterol, mg/dl	231.4 (1.3)	233.6 (2.2)	231.9 (2.2)	228.7 (2.2)	0.2991
Hypertension, %	43.5	36.4	40.6	53.6	<0.0001
Systolic blood pressure, mm Hg	129 (0.6)	126.8 (1.0)	128.2 (1.0)	133.4 (1.0)	<0.0001
Diastolic blood pressure, mm Hg	78.4 (0.3)	78.8 (0.5)	78.1 (0.5)	78.4 (0.5)	0.6726

Abbreviation: TNF-α, tumor necrosis factor alpha.
[a]Numbers in the table shown are mean (standard error) for continuous variable or percentage for categorical variables.
[b]TNF-α receptor 2 tertile cutoffs: Tertile 1 (men: ⩽2043.9 pg/ml, women: ⩽2078.5 pg/ml), Tertile 2 (men: 2044.0–2491.5 pg/ml, women: 2078.6–2544.5 pg/ml), Tertile 3 (men: >2491.5 pg/ml, women: >2544.5 pg/ml).
[c]*P*-value estimated by analysis of variance or χ^2-test as appropriate.

Table 2 | Mean levels of markers of inflammation by level of kidney function at the baseline examination

		Mean (standard error) of the inflammatory marker measured in serum			
Estimated glomerular filtration rate (eGFR) category, ml/min per 1.73 m^2	% Of study population in each eGFR category	C-reactive protein (mg/dl)	Tumor necrosis factor-α receptor 2 (pg/ml)	White blood cell count (per 10^3 cells/mm^3)	Interleukin-6 (pg/ml)
⩾60	84.9	3.9 (0.2)	2454.4 (27.8)	6.7 (0.03)	3.6 (0.3)
45–59	11.7	5.2 (0.4)	3273.2 (67.8)	7.3 (0.09)	4.1 (0.7)
30–44	2.9	7.8 (0.9)	4366.3 (153.8)	7.9 (0.2)	5.1 (1.9)
<30	0.5	12.4 (2.1)	10,449.5 (368.8)	9.1 (0.5)	8.4 (4.6)
P-trend		<0.0001	<0.0001	0.002	0.04

DISCUSSION

In a population-based study of predominantly White subjects from Wisconsin, we found that markers of inflammation, including high-sensitivity CRP, TNF-αR2, WBC count, and IL-6 levels, were positively associated with prevalent CKD at baseline. However, in a longitudinal analysis examining the risk of developing CKD over a 15-year follow-up period, only TNF-αR2, WBC count, and IL-6 levels, not CRP levels, were associated with incident CKD. The strongest and most consistent association was observed for TNF-αR2. These results remained relatively robust after multivariable adjustment of confounders. Furthermore, the results also remained consistent when we used a more specific definition to define CKD.[12] Results from our study further extend the current understanding of the role of inflammatory markers in the pathogenesis of CKD. Elevated CRP may be a marker of heightened inflammatory processes known to be activated secondary to kidney disease.[13–15]

Inflammation is a common feature of end-stage renal disease.[16–18] Several studies have also documented significantly higher levels of markers of inflammation among subjects with CKD,[13,19] and have closely correlated inflammatory biomarker levels to changes in GFR[20–22] among subjects with CKD. In the current study, we found that all markers of inflammation, including high-sensitivity CRP, TNF-αR2, WBC count, and IL-6 levels, were related to prevalent CKD in the cross-sectional analysis. However, CRP was not associated with incident CKD in the longitudinal analysis.

It is currently recognized that CKD is an independent risk factor for cardiovascular disease and mortality.[23,24] It has also been shown that heightened inflammatory processes among

Table 3 | Cross-sectional association between markers of inflammation and the prevalence of chronic kidney disease (CKD)

Inflammatory marker level	No. at risk	CKD cases	Unadjusted odds ratio (95% confidence interval)	Age-sex-adjusted odds ratio (95% confidence interval)	Multivariable-adjusted odds ratio (95% confidence interval)[a]
Serum C-reactive protein (CRP)[b]					
Tertile 1	1370	152	1 (Referent)	1 (Referent)	1 (Referent)
Tertile 2	1379	199	1.35 (1.08–1.69)	1.18 (0.97–1.45)	1.09 (0.89–1.35)
Tertile 3	1375	272	1.98 (1.59–2.45)	1.66 (1.38–2.01)	1.48 (1.22–1.79)
P-trend			<0.0001	0.001	0.012
Serum tumor necrosis factor (TNF)-α receptor 2[c]					
Tertile 1	501	29	1 (Referent)	1 (Referent)	1 (Referent)
Tertile 2	502	44	1.56 (0.96–2.54)	1.37 (0.87–2.18)	1.43 (0.89–2.27)
Tertile 3	500	146	6.71 (4.40–10.23)	3.39 (2.28–5.03)	3.50 (2.35–5.24)
P-trend			<0.0001	<0.0001	<0.0001
Serum white blood cell (WBC) count[d]					
Tertile 1	1378	129	1 (Referent)	1 (Referent)	1 (Referent)
Tertile 2	1375	231	1.95 (1.55–2.46)	1.48 (1.20–1.82)	1.32 (1.06–1.64)
Tertile 3	1371	263	2.30 (1.84–2.99)	1.99 (1.63–2.42)	1.94 (1.60–2.38)
P-trend			<0.0001	<0.0001	<0.0001
Serum interleukin-6[e]					
Tertile 1	501	48	1 (Referent)	1 (Referent)	1 (Referent)
Tertile 2	502	76	1.68 (1.15–2.47)	1.43 (1.01–2.03)	1.22 (0.86–1.76)
Tertile 3	500	95	2.21 (1.53–3.21)	1.86 (1.34–2.58)	1.77 (1.27–2.41)
P-trend			<0.0001	<0.0001	0.002

[a]Adjusted for age (years), sex, education categories (<high school, high school, >high school), smoking (never, former, current), alcohol intake (never, former, current), body mass index (kg/m^2), diabetes (absent, present), glycosylated hemoglobin (%), hypertension (absent, present), mean arterial blood pressure (mm Hg), and serum total cholesterol (mg/dl).
[b]Serum CRP tertile cutoffs (mg/dl): Tertile 1 (men: <1.21 mg/l, women: <1.29), Tertile 2 (men: 1.21–2.95, women: 1.29–3.34), and Tertile 3 (men: >2.95, women: >3.34).
[c]Serum TNF-α receptor 2 tertile cutoffs (pg/ml): Tertile 1 (men: <2084.8, women: <2136.6), Tertile 2 (men: 2084.8–2629.6, women: 2136.6–2715.1), and Tertile 3 (men: >2629.6, women: >2715.1).
[d]Serum white blood cell count tertile cutoffs (per 1000 cells/mm^3): Tertile 1 (men: <6.4, women: <6.2), Tertile 2 (men: 6.4–7.9, women: 6.2–7.9), and Tertile 3 (men: >7.9, women: >7.9).
[e]Serum interleukin-6 tertile cutoffs (pg/ml): Tertile 1 (men: <1.75, women: <1.82), Tertile 2 (men: 1.75–2.90, women: 1.82–3.04), and Tertile 3 (men: >2.90, women: >3.04).

CKD subjects are partly responsible for this higher cardiovascular disease risk.[14] Recent guidelines recommend the use of CRP levels as a cardiovascular risk-stratification tool.[25] Our results suggest that CRP measurement may identify inflammatory processes occurring secondary to kidney disease. CRP measurement may therefore be a risk-stratification tool of cardiovascular risk due to kidney disease.

Several lines of recent evidence also suggest that inflammatory processes may have a role in the development of CKD. First, it is possible that inflammation could be related to CKD development indirectly through its role in comorbid conditions such as diabetes[3] or hypertension.[5] However, to examine this hypothesis, when we additionally adjusted for diabetes and hypertension in the multivariable model in Table 3, there was no change in hazard ratios, suggesting that these conditions are probably not a mediator in our study. Second, elevated circulating CRP levels deposited in the glomerular endothelium may be the initial injury that may then function through several interrelated hypothesized mechanisms, including elevated angiotensin II levels, increased transforming growth factor-β levels, decreased nephron mass, glomerular-capillary hypertension, tubular cell hypertrophy, fibroblast proliferation, increased type IV collagen synthesis, and interstitial inflammation, leading to renal scarring.[7,26] Third, several animal studies also suggest that inflammation may contribute to CKD development. Fattori *et al.*[27] reported that transgenic mice that constitutively express the inflammatory cytokine IL-6 in the liver and secrete it into blood develop progressive kidney disease. Bertani *et al.*[8] reported that rabbits injected with increasing doses of human recombinant TNF-α showed dose-dependent glomerular endothelial cell damage. Rats pretreated with TNF-α and IL-6 showed increasing severity of glomerular injury.[9]

In contrast to animal studies, there is limited evidence on inflammatory processes and the risk of developing CKD from human epidemiological studies.[13,28,29] Previous cross-sectional[18,28,30] and case–control[13] studies have reported an association between inflammatory markers and CKD or reduced kidney function. However, it is known that inflammatory markers are secondarily elevated in subjects with CKD[13–15] and end-stage renal disease,[16–18] reflecting a heightened inflammatory response that occurs subsequent to kidney disease. Therefore, a critical factor required to clarify the direction of the putative inflammation–CKD association is the need for a longitudinal study design among individuals initially free of CKD at baseline. Even though one previous longitudinal study demonstrated an association between CRP levels and CKD, it included subjects with decreased kidney function at baseline.[31] It is possible that the observed association between CRP and serum creatinine may be due to CRP elevations occurring secondary to kidney disease.[13–15]

Table 4 | Longitudinal association between markers of inflammation and 15-year incidence of chronic kidney disease (CKD)

Inflammatory marker level	No. at risk	CKD cases	Unadjusted hazard ratio (95% confidence interval)	Age-sex-adjusted hazard ratio (95% confidence interval)	Multivariable-adjusted hazard ratio 1 (95% confidence interval)[a]	Multivariable-adjusted hazard ratio 2 (95% confidence interval)[a]
Serum C-reactive protein (CRP)[b]						
Tertile 1	960	197	1 (Referent)	1 (Referent)	1 (Referent)	1 (Referent)
Tertile 2	961	234	1.19 (1.00–1.40)	1.13 (0.95–1.33)	1.10 (0.92–1.32)	1.06 (0.89–1.27)
Tertile 3	956	244	1.24 (1.05–1.47)	1.19 (0.97–1.46)	1.14 (0.95–1.37)	1.09 (0.91–1.29)
P-trend			0.001	0.09	0.21	0.42
Serum tumor necrosis factor (TNF)-α receptor 2[c]						
Tertile 1	349	54	1 (Referent)	1 (Referent)	1 (Referent)	1 (Referent)
Tertile 2	350	82	1.51 (1.11–2.06)	1.49 (1.06–2.09)	1.47 (1.01–2.14)	1.40 (0.94–2.09)
Tertile 3	349	132	2.44 (1.85–3.23)	2.68 (1.73–4.15)	2.49 (1.66–3.74)	2.10 (1.55–2.84)
P-trend			<0.0001	<0.0001	<0.0001	<0.0001
Serum white blood cell (WBC) count[d]						
Tertile 1	961	140	1 (Referent)	1 (Referent)	1 (Referent)	1 (Referent)
Tertile 2	960	252	1.80 (1.50–2.17)	1.63 (1.36–1.95)	1.58 (1.29–1.94)	1.41 (1.16–1.70)
Tertile 3	956	293	2.10 (1.76–2.52)	2.04 (1.70–2.45)	1.98 (1.68–2.33)	1.90 (1.59–2.27)
P-trend			<0.0001	<0.0001	0.0011	0.0014
Serum interleukin-6[e]						
Tertile 1	351	51	1 (Referent)	1 (Referent)	1 (Referent)	1 (Referent)
Tertile 2	348	97	1.92 (1.41–2.60)	1.43 (0.98–2.07)	1.39 (0.95–2.03)	1.32 (0.89–1.97)
Tertile 3	349	120	2.37 (1.77–3.17)	1.72 (1.11–2.67)	1.57 (1.04–2.37)	1.45 (0.99–2.14)
P-trend			<0.0001	0.022	0.043	0.073

[a]Multivariable-adjusted hazard ratio 1: adjusted for age (years), sex, education categories (<high school, high school, >high school), smoking (never, former, current), alcohol intake (never, former, current), body mass index (kg/m^2), glycosylated hemoglobin (%), mean arterial blood pressure (mm Hg), and serum total cholesterol (mg/dl); Multivariable-adjusted hazard ratio 2: additionally adjusted for diabetes (absent, present) and hypertension (absent, present).
[b]Serum C-reactive protein cutoffs (mg/dl): Tertile 1 (men: <1.13, women: <1.19), Tertile 2 (men: 1.13–2.60, women: 1.19–2.99), and Tertile 3 (men: >2.60, women: >2.99).
[c]Serum TNF-α receptor 2 tertile cutoffs (pg/ml): Tertile 1 (men: <2043.9, women: <2078.5), Tertile 2 (men: 2043.9–2491.5, women: 2078.5–2544.5), and Tertile 3 (men: >2491.5, women: >2544.5).
[d]Serum white blood cell count tertile cutoffs (per 1000 cells/mm^3): Tertile 1 (men: <6.2, women: <5.8), Tertile 2 (men: 6.2–7.8, women: 5.8–7.3), and Tertile 3 (men: >7.8, women: >7.3).
[e]Serum interleukin-6 tertile cutoffs (pg/ml): Tertile 1 (men: <1.5, women: <1.6 mg/l), Tertile 2 (men: 1.5–2.6, women: 1.6–2.7), and Tertile 3 (men:>2.6, women: >2.7).

In this context, Erlinger *et al.*[32] and Bash *et al.*[33] analyzed longitudinal data from the NHANES II follow-up study and the Atherosclerosis Risk in Communities Study, respectively, and showed that among subjects free of CKD at baseline, elevated WBC count, a nonspecific marker of inflammation, independently predicted the risk of developing CKD.

In the current study, we also found that elevated WBC count was associated with incident CKD. However, we also measured specific markers of inflammation, including high-sensitivity CRP, TNF-αR2, and IL-6 levels, and examined their association with the risk of developing incident CKD over a 15-year follow-up period in the longitudinal analysis. We found that only TNF-αR2 and IL-6 levels, not CRP levels, predicted the risk of developing incident CKD. In contrast, CRP, TNF-αR2, and IL-6 levels were all associated with prevalent CKD in the cross-sectional analysis. Finally, the fact that we obtained consistent results when we repeated the main analysis defining CKD using a more specific definition recently suggested by Bash *et al.*[12] suggests that these results are not likely to be due to chance.

Our results suggest that, similar to animal studies,[8,9] inflammatory mechanisms may be involved in the etiology of CKD in humans also and that TNF-αR2 level elevations are an early marker of these mechanisms. The observed lack of association between CRP levels and incident CKD may be due to several possible reasons. Elevation of CRP levels may be a marker/intermediary effect of antecedent inflammatory processes (e.g., TNF-α-mediated inflammation). As a result, CRP elevations may be evident only in relatively later stages of kidney disease development, and therefore not useful for early prediction of CKD. Alternatively, it is also possible that any CRP–CKD association may entirely be explained by confounders that were adjusted in our multivariable model, such as age, gender, body mass index, diabetes, or hypertension. Finally, if the true CRP association is relatively weak, statistical power could be lower than for TNF-αR2.

Strengths of this study include its population-based sample, high participation rate, use of standardized protocols for exposure and outcome measurement, and the availability of specific markers of inflammation, including CRP and TNF-αR2 levels. We had validated measures of kidney function, including eGFR, using standardized serum creatinine as per the recent National Kidney Disease Education Program guidelines.[34] A main study limitation is the lack of information on a marker of kidney injury such as 24-h urine protein or urinary albumin/creatinine ratio. Second, similar to previously published studies using stored baseline blood,[35] we measured serum TNF-αR2 levels in the current study as a measure of TNF-α-related inflammation. Third, as our study sample is composed of predominantly White participants,

our results may not be generalizable to other racial–ethnic groups. Fourth, the markers of inflammation that we studied may be affected by intervening episodes of acute renal failure/acute kidney injury for which we did not have data available. As acute renal failure/acute kidney injury is also a risk factor for incident CKD, it is possible that the observed association in our study is be explained by this unmeasured confounder.

In conclusion, results from this study support the hypothesis of an association between elevated markers of inflammation—including TNF-αR2, WBC count, and IL-6 levels—and the risk of developing CKD, suggesting a role for inflammatory mechanisms in the etiology of CKD. A practical application of our findings is that, if replicated in other studies, these markers may be a potential future way of assessing subjects who are more likely to develop CKD.

MATERIALS AND METHODS

The Beaver Dam Chronic Kidney Disease Study is a prospective cohort study of risk factors for CKD. The study was designed as an ancillary study within the population-based Beaver Dam cohort. The exposure, outcome, and covariate information for Beaver Dam Chronic Kidney Disease Study were obtained by combining (1) the newly measured biomarker data, including serum creatinine and markers of inflammation, measured from stored blood in two related prospective cohort studies that followed the same set of study subjects of the Beaver Dam cohort, the Beaver Dam Eye study,[36,37] and the Epidemiology of Hearing Loss Study,[38,39] and (2) questionnaire, physical examination, and laboratory measurement data already collected in these studies. The methods used to identify and describe the Beaver Dam population have appeared in previous reports.[36–39]

In brief, a private census of the population of Beaver Dam, Wisconsin, was carried out from September 1987 to May 1988 to identify all residents in the city or township of Beaver Dam who were in the age group of 43–84 years. Of the 5924 eligible individuals (98% Caucasians), 4926 (83.1%) participated in the baseline examination of Beaver Dam Eye study between 1 March 1988 and 14 September 1990. Comparisons between participants and non-participants at the time of the baseline examination have appeared elsewhere.[37] The Beaver Dam Eye study participants alive as on 1 March 1993, were eligible for the baseline examination for Epidemiology of Hearing Loss Study ($n = 4541$), which occurred at the time of the 5-year follow-up visit for the eye study.

Therefore, the assembled Beaver Dam Chronic Kidney Disease Study data involved a baseline examination from 1988 to 1990, a 5-year follow-up examination from 1993 to 1995, a 10-year follow-up examination from 1998–2000, and a 15-year follow-up examination from 2003–2005. Approximately 73% of the surviving Beaver Dam participants were studied at the follow-up examinations. Written informed consent was obtained from each subject at each examination. The study was approved by the Human Subjects Committee of the University of Wisconsin School of Medicine and Public Health, Madison, WI.

The current paper presents two sets of analyses: (1) a cross-sectional analysis with prevalent CKD as the outcome of interest and (2) a longitudinal analysis with 15-year incident CKD as the outcome of interest. The sample sizes were different for each inflammatory biomarker, as high-sensitivity CRP and WBC count were available on all baseline cohort subjects ($n = 4880$), whereas TNF-αR2 and IL-6 were measured only in a random sample of the baseline cohort ($n = 1789$). The sample construction for the cross-sectional and longitudinal analysis are presented in Figures 1 and 2, respectively.

Exposure ascertainment

The baseline and follow-up examinations included measurement of weight, height, systolic, and diastolic blood pressure by trained observers, and administering a standardized questionnaire that collected information regarding participants' demographic characteristics, details regarding cigarette smoking, alcohol intake, medical histories, and medications taken, including physician-diagnosed diabetes, hypertension, or cardiovascular disease. Non-fasting blood specimens were obtained for measurement of plasma glucose, glycosylated hemoglobin, serum total cholesterol, and high-density lipoprotein cholesterol.

Age was defined as the participants' age at the time of the baseline examination. Education was categorized as less than high school, high school, or beyond high school. Body mass index was defined as participants' weight in kilograms divided by their height in meters squared. Hypertension was defined as a systolic blood pressure of 140 mm Hg or higher, and/or a diastolic blood pressure 90 mm Hg or higher, and/or the combination of self-reported hypertension diagnosis by a physician, and use of antihypertensive medications. Persons were defined as having diabetes mellitus if they had a history of diabetes diagnosis by a physician and were treated with insulin, oral hypoglycemic agents or diet, or were newly classified as having diabetes based on the presence of a casual blood sugar value $\geqslant 200$ mg/dl (11.1 mmol/l) or a glycosylated hemoglobin value that was >2 standard deviations above the mean for a given age/gender group (for those in the age group of 43–54 years, men $>9.5\%$ and women $>9.6\%$; for those in the age group of 55–64 years, men $>9.4\%$ and women $>10.0\%$; for those in the age group of 65–74 years, men $>9.6\%$ and women $>9.6\%$; and for those $\geqslant 75$ years, men $>9.5\%$ and women $>9.6\%$).

At the baseline and follow-up examinations, additional blood samples were stored in freezers at $-80\,^{\circ}C$, until the time of laboratory analysis. Quality control samples were routinely frozen with study participant samples. Baseline frozen samples were analyzed for high-sensitivity CRP, TNF-αR2, and IL-6 levels. High-sensitivity CRP was measured in serum in all eligible baseline study subjects ($n = 4880$) using latex-particle-enhanced immunoturbidimetric assay kit (Kamiya Biomedical Company, Seattle, WA) and read on the Roche/Hitachi 911 (Roche Diagnostics, Indianapolis, IN). The reference range is 0–0.5 mg/dl. The inter-assay coefficient of variability range was 4.5%. TNF-αR2 levels were measured in serum in a random sample of baseline cohort participants ($n = 1789$) using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay QuantiKine kit from R&D Systems (Minneapolis, MN). The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA). The inter-assay coefficient of variability was 3.5–5.1%. IL-6 level was measured in serum using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay QuantiKine High Sensitivity kit from R&D Systems. The intensity of the color is measured on a SpectraMax spectrophotometer (Molecular Devices). The inter-assay coefficient of variability range reported was 6.5–9.6%. WBC count was determined using a Coulter counter method. Reliability coefficients, based on blind replicate control data, ranged from 0.96 to 1.00.

Outcome of interest: chronic kidney disease

Serum was stored without preservative at −80 °C in cryogenic vials with O-rings for up to 17 years, until the vials were shipped on dry ice to the University of Minnesota laboratory for carrying out the analyses reported in this study. Creatinine level was measured in serum from all study examinations by reflectance spectrophotometry on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Rochester, NY), consistent with the current National Kidney Disease Education Program recommendations for standardizing serum creatinine measurement.[34] The laboratory coefficient of variability was 2.2%.

Glomerular filtration rate was estimated from serum creatinine using the re-expressed Modification of Diet in Renal Disease equation is defined as follows: eGFR = 175 × (serum creatinine in mg/dl)$^{-1.154}$ × age$^{-0.203}$ × (0.742 for women). CKD was defined as an eGFR of <60 ml/min per 1.73 m^2, based on the US National Kidney Foundation Kidney Disease Outcome Quality Initiative working group definition,[40] and the Kidney Disease Improving Global Outcomes definition.[41] Prevalent CKD was defined as the presence of CKD at the baseline examination. Death due to CKD ($n = 6$) was defined as the underlying cause of death reported as chronic renal disease (ICD-9 codes 581–583 or 585–588); hypertensive renal disease (ICD-9 code 403); hypertensive heart and renal disease (ICD-9 code 404); unspecified disorder of kidney and ureter (ICD-9 code 593.9); diabetes with renal manifestations (ICD-9 code 250.3); kidney transplant, renal dialysis, or adjustment/fitting of catheter (ICD-9 codes V42.0, V45.1 or V56); and hemodialysis (ICD-9 code 39.95) or peritoneal dialysis (ICD-9 code 54.98), without acute renal failure (ICD-9 codes 584, 586, 788.9, and 958.5). Incident CKD was defined as the new development of CKD at the 5-, 10- or 15-year follow-up examination or subjects who died because of CKD among subjects free of CKD at baseline.

Statistical methods

We were interested in the association between markers of inflammation (serum CRP, TNF-αR2, WBC count, and IL-6 levels) and CKD. We performed two sets of analyses; the first set examined the cross-sectional association between these inflammatory markers and prevalent CKD, and the second set examined the longitudinal association with 15-year incident CKD. We categorized inflammatory markers into tertiles for the analysis. We used the χ^2-test to compare categorical variables, and analysis of variance to compare continuous variables between increasing categories of TNF-αR2. We used multivariable proportional hazards models with discrete handling of ties to determine the Hazards ratio and 95 percent confidence interval (CI) of 15-year incident CKD, controlling simultaneously for potential confounders. Age and sex are well-known confounders for the inflammation–CKD association. Therefore, we choose to present our results starting from the age- and sex-adjusted model rather than the unadjusted model. Subsequently, we are presenting two nested proportional hazards models in this paper: an age- (years) and sex-adjusted model, and a multivariable-adjusted model, including additional adjustment for education (<high school, high school, >high school), smoking (never, former, current), alcohol intake (never, former, current), body mass index (kg/m^2), diabetes mellitus (absent, present), glycosylated hemoglobin level (%), hypertension (absent, present), mean arterial blood pressure (mm Hg), and serum total cholesterol (mg/dl). Linear trends across tertiles were assessed by including tertile-specific median inflammatory biomarker values as a continuous variable in the proportional hazards regression models.

We also performed the following supplementary analyses. First, to examine whether the positive association observed between increasing TNF-αR2 levels and 15-year incident CKD in the longitudinal analysis was independent of other inflammatory markers (CRP, WBC count, and IL-6 levels), we additionally adjusted for levels of these markers as a continuous variable in the multivariable model. Second, to rule out the possibility of reverse causality, we repeated the main analysis after excluding the CKD cases occurring during the initial 5 years of follow-up. Finally, we repeated the main analyses using a more specific definition of CKD by Bash *et al.*,[12] defined as both an eGFR falling below 60 ml/min per 1.73 m^2 (our main definition) and a decrease in eGFR of at least 25% over the follow-up period.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

All authors contributed to the intellectual development of this paper. AS had the original idea for the study, analyzed the data, wrote the first draft paper, and is the guarantor. LS analyzed the data. RK, BEKK, JC, PM, KJC, CRS, PCB, KEL, and FJN provided statistical expertise, critical corrections to the manuscript, and were involved in manuscript revisions. AS, RK, BEK, and KJC procured funding for the study and they, with CRS, supervised data collection.

Ethics approval: This study followed the recommendations of the Declaration of Helsinki and was approved by the Human Subjects Committee of the University of Wisconsin School of Medicine and Public Health, Madison, WI. Written, informed consent was obtained from all participants.

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