Association between Time-Updated Eosinophil Counts and Progression of CKD

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Article

Keywords: eosinophil, chronic kidney disease, mortality, cardiovascular events, marginal structural model

Posted Date: September 2nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2003296/v1

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Abstract

Patients with chronic kidney disease (CKD) have high blood eosinophil count but its clinical implication is uncertain. Since eosinophils may induce tubulointerstitial injury and arteriosclerosis, eosinophilia might be related to poor clinical outcomes. This retrospective cohort study included 2,877 patients whose estimated glomerular filtration rate (eGFR) was 10–60 mL/min/1.73 m². The exposure was time-updated blood eosinophil counts. The outcomes were 1) initiation of renal replacement therapy (RRT) and 2) cardiovascular events and mortality. We analyzed the associations between eosinophil counts and outcomes using marginal structural models (MSM). Over a median follow-up of 6.5 years, eosinophil counts were measured a median of 22 times per patient (4 times a year per patient). There was a negative correlation between eosinophil count and eGFR. In total, 433 patients initiated RRT, 275 developed cardiovascular events, and 165 died. In MSM, higher eosinophil counts (≥ 289/µL) showed a 1.83-fold (95% confidence interval:1.33–2.51) higher rate of RRT initiation than lower eosinophil counts after adjustment for time-dependent confounders. Higher eosinophil counts were also associated with a higher rate of cardiovascular events and mortality in MSM (hazard ratio, 1.71 [95% confidence interval:1.30–2.25]). In conclusion, patients with CKD who had higher eosinophil counts showed worse kidney outcome.

Introduction

Eosinophils are multifunctional leukocytes involved in an array of pathological processes. Besides their well-known roles in allergic reactions, parasite defense, and autoimmune diseases, eosinophils are also implicated in atherosclerosis. Marx et al. showed that activated eosinophils in atherosclerotic lesions accelerate plaque formation in concert with platelets by secreting eosinophilic granule proteins and extracellular traps. Population-based cohort studies reported increased cardiovascular risks among those with higher levels of plasma eosinophilic cationic protein (ECP), a marker of eosinophil activity and degranulation.

Patients with advanced chronic kidney disease (CKD) have high blood eosinophil count although its clinical implication is uncertain. In addition to their proatherogenic property, eosinophils might also contribute to the progression of kidney disease. For example, renal complications sometimes develop in idiopathic hypereosinophilic syndrome, where tubulointerstitial infiltration of eosinophils is typically observed. Eosinophilic granulomatosis with polyangiitis, characterized by the interstitial infiltration of eosinophils, is also evidentiary to the involvement of eosinophils in kidney injury. Furthermore, interstitial eosinophilic aggregates are found in common kidney diseases, such as diabetic nephropathy, IgA nephropathy, and membranous nephropathy, which are related to interstitial fibrosis and inflammation. Since there is a positive correlation between eosinophil counts in the blood and renal interstitium, we hypothesized that increased blood eosinophil counts in patients with CKD reflect eosinophilic inflammation in the kidney and thus indicate a risk of CKD progression. In the current study, we examined the association between time-updated blood eosinophil counts and the risk of kidney failure among patients with advanced CKD.

Methods

Ethical considerations

This study was conducted in accordance with the principles of the Declaration of Helsinki. The Ethics Committee of Osaka University Hospital approved the study protocol and waived the need for a written informed consent given the retrospective nature of the study (no: 20352, 22047).

Study design and participants
This retrospective cohort study included all patients referred to the outpatient department of nephrology at Osaka University Hospital from January 2005 to January 2018 who met the following inclusion criteria: 1) aged 20 years or older, 2) an estimated glomerular filtration rate (eGFR) of 10–60 mL/min/1.73 m², and 3) not receiving renal replacement therapy (RRT). Patients were excluded if they 1) were followed up for < 1 year or 2) received corticosteroids.

Follow-up period was from the first visit to the hospital to death, RRT initiation, loss to follow-up, or February 28th, 2019, whichever occurred first.

Data collection

The detailed methods have been described elsewhere. Demographics and comorbidities were extracted from a chart review of patients’ electronic medical records by nephrologists. These included age, sex, body mass index (BMI), blood pressure, diabetes mellitus (DM), cardiovascular comorbidities, chronic respiratory diseases (chronic obstructive pulmonary disease and bronchial asthma), a prior history of arterial catheterization (cardiac catheterization and endovascular treatment for peripheral artery diseases and carotid artery stenosis), and cholesterol embolism. Cardiovascular comorbidities included coronary artery disease, congestive heart failure, valvular heart disease, aortic disease, and stroke (cerebral infarction or intracranial hemorrhage).

Time-series data on laboratory measurements and prescriptions were collected using an automated data extraction system of Osaka University Hospital. Laboratory data included serum albumin, creatinine, sodium, potassium, C-reactive protein (CRP), hemoglobin, white blood cell (WBC) counts, and urinary protein-to-creatinine ratio (UPCR). eGFR was calculated using the equation for the Japanese population. The prescription data included loop diuretics, thiazide diuretics, mineralocorticoid receptor antagonists (MRAs), angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), nonsteroidal anti-inflammatory drugs (NSAIDs), proton pump inhibitors (PPIs), histamine H₂ receptor antagonists (H₂ blockers), and corticosteroids. We assessed the number of prescription drugs, not limited to those mentioned above. The time-series data were collected at a monthly interval during the study period.

We collected information regarding arterial catheterization performed during follow-up (cardiac catheterization and endovascular treatment for peripheral artery diseases and carotid artery stenosis); these procedures may have contributed to the development of eosinophilia via cholesterol embolism. These data were obtained from diagnostic procedure combination (DPC) codes.

Exposure

The exposure was time-updated blood eosinophil counts (per µL), which were collected at a monthly interval during the study period. Blood eosinophil counts were calculated by multiplying the total WBC count by the percentage of eosinophils as measured using an automated WBC differential counter. Blood eosinophil counts were categorized into quartiles.

Study outcomes

The study outcome was RRT initiation, defined as the initiation of chronic dialysis or kidney transplantation. We additionally evaluated cardiovascular outcomes, which were a composite of myocardial infarction, stroke, hospitalization for heart failure, and mortality. The dates of these clinical events were ascertained by a chart review of the patients’ electronic medical records by nephrologists.

A post-hoc analysis of a randomized controlled trial of oral carbon adsorbent

To explore the involvement of uremic toxins in elevated eosinophil counts in CKD, we analyzed the data in a previous randomized controlled trial of the oral carbon adsorbent AST-120, which reduces serum uremic toxin levels such as
Indoxyl sulfate. This two-year, open-label, randomized, controlled trial enrolled 125 patients with stages 3–4 CKD. Among them, 123 were randomized to either receive AST-120 (6 g/day) or not, in a 3:2 ratio. In this post-hoc analysis, data on eosinophil counts at baseline, 3, 6, and 12 months were added to the original dataset.

**Statistical analyses**

The relationship between eosinophil counts and eGFR at baseline was depicted using a restricted cubic spline curve with three knots (10th, 50th, and 90th percentiles of eGFR).

The multivariable association between log-transformed eosinophil counts and covariates was assessed by a linear regression analysis with robust standard errors. The following variables were included: age, sex, BMI, systolic blood pressure, DM, cardiovascular comorbidities, a prior history of arterial catheterization, chronic respiratory diseases, ACEIs/ARBs, loop diuretics, thiazide diuretics, MRAs, PPIs, H₂ blockers, NSAIDs, number of drugs prescribed, hemoglobin, albumin, eGFR, CRP, UPCR, and WBC.

In the post-hoc analysis of the randomized trial of AST-120, eosinophil counts were compared between the AST-120 and control groups using a linear mixed-effects model for repeated measures with an unstructured covariance matrix.

To analyze the longitudinal relationship between time-updated blood eosinophil counts and kidney outcomes, time-dependent confounding should be considered. This is because eosinophil counts increase as kidney function declines. As a result, time-dependent confounding could occur owing to a potential bidirectional relationship between eosinophil counts and kidney function in terms of the development of kidney failure. In order to appropriately account for time-dependent confounding, we used a marginal structural model (MSM). We also performed baseline Cox model, time-average Cox model, and group-based trajectory model (Fig. 1).

1) Baseline Cox model

Association between baseline eosinophil quartiles and outcomes was analyzed using multivariate Cox proportional hazards models. The following baseline covariates were adjusted in this model: age, sex, BMI, systolic blood pressure, DM, cardiovascular comorbidities, chronic respiratory diseases, a prior history of arterial catheterization and cholesterol embolism, hemoglobin, albumin, eGFR, sodium, potassium, CRP, WBC, UPCR, loop diuretics, thiazide diuretics, MRAs, ACEIs, ARBs, NSAIDs, PPIs, and H₂ blockers. The proportional hazards assumption was checked graphically based on the scaled Schoenfeld residuals.

2) Time-average Cox model

The average eosinophil count during the first 12 months of follow-up was calculated for each patient. Association between time-average eosinophil quartiles and outcomes was analyzed using a multivariate Cox proportional hazards model adjusted for the same covariates as in the baseline model. In this model, the onset of survival time was set at 12 months.

3) Group-based trajectory model

Group-based trajectory model was used to assess the association between eosinophil count trajectories during the first 12 months and subsequent rates of outcomes (STATA command, traj). All available data on eosinophil counts during the first 12 months were used to identify eosinophil count trajectories. In this analysis, the eosinophil counts were log-transformed to normalize their distribution.

The group-based trajectory model is a method of data clustering that assumes that a population is composed of a mixture of distinct groups characterized by their longitudinal trajectories. Potential trajectory groups were estimated...
from individual longitudinal eosinophil data, using the maximum likelihood estimation method based on the finite mixture model theorem. The patients were divided into one of the trajectory groups according to their estimated probability of group membership. We selected the optimal number of trajectory groups, as well as a function of each trajectory, based on the Bayesian information criterion (BIC), with at least 5% of all patients being in the smallest group.

After deriving the eosinophil trajectory groups, multivariate Cox proportional hazards models were used to analyze the association between the trajectory groups and outcomes, adjusting for the same covariates as in the baseline model. In this model, the onset of survival time was set at 12 months.

4) MSM

MSM was employed to 1) assess the time-varying eosinophil counts throughout the study period and 2) deal with time-dependent confounding between eosinophil counts and eGFR.

MSM is a statistical method that can account for time-dependent confounding. In the current study, eGFR was considered to be the main time-dependent confounder because it influenced both exposure (eosinophil counts) and renal outcomes, while being possibly affected by previous eosinophil counts. We derived time-varying inverse probability weights (IPWs) from the inverse probability of treatment weights (IPTWs) and the inverse probability of censoring weights (IPCWs). IPTWs were the reciprocal of the predicted probability of each patient having their own exposure history (i.e., high eosinophil count or not). The probability was predicted by a logistic regression model at each of the 1-month follow-up periods, conditional on both baseline and time-dependent covariates, as described below. Two different definitions of high eosinophil counts were adopted: 1) eosinophil count $\geq 289/\mu$L (the top 25th percentile in our cohort) and 2) eosinophil count $\geq 500/\mu$L. Similarly, IPCWs were the reciprocal of the probability of being uncensored, as predicted by a logistic regression model, conditional on both baseline and time-dependent covariates. IPTWs and IPCWs were stabilized by multiplying them with the predicted probabilities based on baseline covariates alone. The IPWs were the product of the stabilized IPTWs and IPCWs, calculated at baseline and for each month. The IPWs were truncated at the 1st and 99th percentiles to reduce the influence of extreme weight values.

Baseline covariates included were the same as in the baseline model. Time-dependent covariates included arterial catheterization performed during follow-up, hemoglobin, albumin, eGFR, sodium, potassium, CRP, UPCR, loop diuretics, thiazide diuretics, MRAs, ACEIs, ARBs, NSAIDs, PPIs, H2 blockers, and corticosteroids.

MSM created “pseudo-populations” using IPWs, comparing the rate of events if all patients had been continuously exposed to high eosinophil counts with the risk of events if they had never been exposed to it. In MSM, there was no association between measured time-dependent confounders and future exposure. We estimated the hazard ratio (HR) and 95% confidence interval (CI) using an IPW-weighted pooled logistic regression model that produced equivalent estimates to the Cox proportional hazards model.

Effect modification was evaluated by incorporating cross-product terms between eosinophil counts and a priori specified baseline covariates into the MSM, including age (< 70 vs. $\geq 70$), sex, BMI (< 22 vs. $\geq 22$), systolic blood pressure (< 130 vs. $\geq 130$ mmHg), DM, cardiovascular comorbidities, hemoglobin (< 12.4 vs. $\geq 12.4$ g/dL), albumin (< 3.8 vs. $\geq 3.8$ g/dL), CKD stage (stage 3 vs. stage 4–5), UPCR (< 1.0 vs. $\geq 1.0$ g/gCr), and ACEIs/ARBs use.

Missing data at baseline were imputed using the multiple imputations by chained equation method based on all baseline covariates. Continuous variables with missing data (BMI, systolic blood pressure, eGFR, hemoglobin, sodium, potassium, UPCR, albumin, and CRP) were imputed based on linear regression imputation. We created ten imputed datasets that were analyzed separately and combined using Rubin’s rules. Missing data during follow-up were imputed using the last-observation-carried-forward method.
Two sensitivity analyses were conducted. First, the association between eosinophil count and RRT initiation was assessed after excluding patients with chronic respiratory diseases or cholesterol embolism. Second, we reanalyzed MSM after excluding patients who were followed up for less than three months.

Statistical analyses were performed using Stata/IC software (version 16.0; Stata Corp, College Station, TX, USA).

**Results**

**Study population**

Among 2,889 patients who met the inclusion criteria, 2,877 (99%) had available eosinophil count data (Figure S1). Over the median follow-up period of 6.5 years (interquartile range, 3.5–9.9), eosinophil count was measured a median of 22 (interquartile range, 7–46) times per patient (4 [interquartile range, 2–6] times a year per patient). The clinical characteristics according to eosinophil quartiles are presented in Table 1. Patients in the highest eosinophil quartile were more likely to be men and to have diabetes mellitus, cardiovascular comorbidities, a lower eGFR, and a higher UPCR. There was a monotonic negative correlation between eosinophil count and eGFR (Fig. 2).
### Table 1
Baseline characteristics according to eosinophil counts quartiles

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total</th>
<th>Missing data</th>
<th>Q1: &lt; 90</th>
<th>Q2: 90–170</th>
<th>Q3: 170–289</th>
<th>Q4: &gt; 289</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>63 (14)</td>
<td>0</td>
<td>63 (14)</td>
<td>64 (14)</td>
<td>63 (14)</td>
<td>63 (16)</td>
<td>0.99</td>
</tr>
<tr>
<td>Male</td>
<td>1,873 (65%)</td>
<td>0</td>
<td>356 (52%)</td>
<td>435 (64%)</td>
<td>509 (69%)</td>
<td>573 (75%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1,173 (41%)</td>
<td>0</td>
<td>243 (35%)</td>
<td>264 (39%)</td>
<td>331 (45%)</td>
<td>335 (44%)</td>
<td>0.002</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23 (4)</td>
<td>84 (3%)</td>
<td>22 (4)</td>
<td>23 (4)</td>
<td>23 (4)</td>
<td>24 (4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>131 (21)</td>
<td>108 (4%)</td>
<td>130 (21)</td>
<td>132 (20)</td>
<td>131 (20)</td>
<td>131 (21)</td>
<td>0.56</td>
</tr>
<tr>
<td>Cardiovascular comorbidities</td>
<td>512 (18%)</td>
<td>0</td>
<td>78 (11%)</td>
<td>118 (17%)</td>
<td>136 (18%)</td>
<td>180 (23%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Prior history of catheterization</td>
<td>415 (14%)</td>
<td>0</td>
<td>71 (10%)</td>
<td>89 (13%)</td>
<td>106 (14%)</td>
<td>149 (19%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Chronic respiratory diseases</td>
<td>43 (1%)</td>
<td>0</td>
<td>7 (1%)</td>
<td>6 (1%)</td>
<td>9 (1%)</td>
<td>21 (3%)</td>
<td>0.005</td>
</tr>
<tr>
<td>ACEIs/ARBs</td>
<td>485 (17%)</td>
<td>0</td>
<td>83 (12%)</td>
<td>103 (15%)</td>
<td>124 (17%)</td>
<td>175 (23%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Loop diuretics</td>
<td>299 (10%)</td>
<td>0</td>
<td>48 (7%)</td>
<td>63 (9%)</td>
<td>68 (9%)</td>
<td>120 (16%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Thiazide diuretics</td>
<td>101 (4%)</td>
<td>0</td>
<td>11 (2%)</td>
<td>24 (4%)</td>
<td>30 (4%)</td>
<td>36 (5%)</td>
<td>0.001</td>
</tr>
<tr>
<td>MRAs</td>
<td>176 (6%)</td>
<td>0</td>
<td>29 (4%)</td>
<td>41 (6%)</td>
<td>39 (5%)</td>
<td>67 (9%)</td>
<td>0.001</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>63 (2%)</td>
<td>0</td>
<td>16 (2%)</td>
<td>12 (2%)</td>
<td>24 (3%)</td>
<td>11 (1%)</td>
<td>0.59</td>
</tr>
<tr>
<td>PPIs</td>
<td>276 (10%)</td>
<td>0</td>
<td>52 (8%)</td>
<td>56 (8%)</td>
<td>61 (8%)</td>
<td>107 (14%)</td>
<td>0.001</td>
</tr>
<tr>
<td>H₂ blockers</td>
<td>285 (10%)</td>
<td>0</td>
<td>68 (10%)</td>
<td>72 (11%)</td>
<td>73 (10%)</td>
<td>72 (9%)</td>
<td>0.65</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>12.3 (2.1)</td>
<td>1 (0%)</td>
<td>12.1 (2.0)</td>
<td>12.5 (2.0)</td>
<td>12.5 (2.1)</td>
<td>12.2 (2.1)</td>
<td>0.68</td>
</tr>
<tr>
<td>Sodium, mEq/L</td>
<td>140 (3)</td>
<td>466 (16%)</td>
<td>140 (3)</td>
<td>140 (3)</td>
<td>140 (3)</td>
<td>139 (3)</td>
<td>0.002</td>
</tr>
<tr>
<td>Potassium, mEq/L</td>
<td>4.4 (0.5)</td>
<td>356 (12%)</td>
<td>4.4 (0.6)</td>
<td>4.4 (0.5)</td>
<td>4.5 (0.5)</td>
<td>4.5 (0.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>3.8 (0.6)</td>
<td>695 (24%)</td>
<td>3.8 (0.6)</td>
<td>3.9 (0.6)</td>
<td>3.8 (0.6)</td>
<td>3.7 (0.6)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data presented as mean (standard deviation), number (%), or median [25th–75th]

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; MRA, mineralocorticoid receptor antagonists; NSAIDs, non-steroidal anti-inflammatory drugs; PPIs, proton pump inhibitors; H₂ blockers, histamine H₂ receptor antagonists; eGFR, estimated glomerular filtration rate; UPCR, urinary protein-to-creatinine ratio
<table>
<thead>
<tr>
<th>Eosinophil counts quartiles: range (/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR, ml/min/1.73m²</td>
</tr>
<tr>
<td>37(14) 50 (2.7%)</td>
</tr>
<tr>
<td>39(14) 38(14) 37(14) 35(14)</td>
</tr>
<tr>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C-reactive protein, mg/dL</td>
</tr>
<tr>
<td>0.1[0.0-0.9] 760 (26%)</td>
</tr>
<tr>
<td>0.1[0.0-0.7] 0.1[0.0-0.7] 0.1[0.0-1.0]</td>
</tr>
<tr>
<td>0.2[0.0-1.1] &lt; 0.001</td>
</tr>
<tr>
<td>UPCR, g/gCre</td>
</tr>
<tr>
<td>0.7[0.0-2.4] 1070 (37%)</td>
</tr>
<tr>
<td>0.4[0.0-1.8] 0.6[0.0-2.2] 0.8[0.0-2.5]</td>
</tr>
<tr>
<td>0.9[0.0-2.7] &lt; 0.001</td>
</tr>
<tr>
<td>White blood cells, ×10³/µL</td>
</tr>
<tr>
<td>7.2(3.4) 0 6.7(3.5) 6.4(2.2) 7.3(3.5)</td>
</tr>
<tr>
<td>8.3(3.7) &lt; 0.001</td>
</tr>
</tbody>
</table>

Data presented as mean (standard deviation), number (%), or median [25th–75th]

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; MRA, mineralocorticoid receptor antagonists; NSAIDs, non-steroidal anti-inflammatory drugs; PPIs, proton pump inhibitors; H₂ blockers, histamine H₂ receptor antagonists; eGFR, estimated glomerular filtration rate; UPCR, urinary protein-to-creatinine ratio

Factors associated with higher eosinophil counts

Eosinophil counts were positively associated with male, BMI, cardiovascular comorbidities, chronic respiratory diseases, ACEIs/ARBs use, and WBC, and negatively correlated with eGFR (Table 2).
Table 2
Multivariable linear regression analysis for the association between log-transformed eosinophil counts and clinical factors

<table>
<thead>
<tr>
<th>Variables</th>
<th>β [95% CI]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per 10 years increase</td>
<td>-0.01 [-0.04, 0.02]</td>
<td>0.52</td>
</tr>
<tr>
<td>Male</td>
<td>0.34 [0.24, 0.43]</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI, per 1 kg/m² increase</td>
<td>0.02 [0.01, 0.03]</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SBP, per 10 mmHg increase</td>
<td>-0.01 [-0.03, 0.01]</td>
<td>0.37</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>-0.02 [-0.11, 0.06]</td>
<td>0.58</td>
</tr>
<tr>
<td>Cardiovascular comorbidities</td>
<td>0.22 [0.11, 0.32]</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Prior history of catheterization</td>
<td>0.02 [-0.10, 0.14]</td>
<td>0.74</td>
</tr>
<tr>
<td>Chronic respiratory diseases</td>
<td>0.40 [0.08, 0.71]</td>
<td>0.01</td>
</tr>
<tr>
<td>ACEIs/ARBs use</td>
<td>0.17 [0.07, 0.26]</td>
<td>0.001</td>
</tr>
<tr>
<td>Loop diuretics use</td>
<td>0.06 [-0.09, 0.20]</td>
<td>0.46</td>
</tr>
<tr>
<td>Thiazide diuretics use</td>
<td>0.07 [-0.10, 0.24]</td>
<td>0.40</td>
</tr>
<tr>
<td>MRAs use</td>
<td>0.01 [-0.16, 0.18]</td>
<td>0.91</td>
</tr>
<tr>
<td>PPIs use</td>
<td>-0.01 [-0.16, 0.13]</td>
<td>0.86</td>
</tr>
<tr>
<td>H₂ blockers use</td>
<td>-0.11 [-0.23, 0.02]</td>
<td>0.09</td>
</tr>
<tr>
<td>NSAIDs use</td>
<td>-0.07 [-0.31, 0.16]</td>
<td>0.54</td>
</tr>
<tr>
<td>Number of drugs, per 1 drug increase</td>
<td>-0.003 [-0.01, 0.01]</td>
<td>0.62</td>
</tr>
<tr>
<td>Hemoglobin, per 1 g/dL increase</td>
<td>0.01 [0.02, 0.03]</td>
<td>0.58</td>
</tr>
<tr>
<td>Albumin, per 1 g/dL increase</td>
<td>0.03 [0.08, 0.14]</td>
<td>0.60</td>
</tr>
<tr>
<td>eGFR, per 10 ml/min/1.73 m² increase</td>
<td>-0.07 [-0.11, -0.04]</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C-reactive protein, per 1 mg/dL increase</td>
<td>-0.01 [-0.03, 0.01]</td>
<td>0.46</td>
</tr>
<tr>
<td>UPCR, per 1 g/gCre increase</td>
<td>0.01 [-0.01, 0.03]</td>
<td>0.32</td>
</tr>
<tr>
<td>White blood cells, per 1000/µL increase</td>
<td>0.05 [0.03, 0.07]</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; BMI, body mass index; SBP, systolic blood pressure; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; MRA, mineralocorticoid receptor antagonist; PPI, proton pump inhibitor; H₂ blockers, histamine H₂ receptor antagonist; NSAID, non-steroidal anti-inflammatory drug; eGFR, estimated glomerular filtration rate; UPCR, urinary protein-to-creatinine ratio

Baseline and time-average Cox models

RRT was initiated in 433 patients (2.1 per 100 patient-years; 95% CI, 1.9 to 2.3). In the baseline Cox model, there was a dose-dependent association between eosinophil quartiles and the rate of RRT initiation (Table 3). Patients in the highest eosinophil quartile had a 2.12-fold (95% CI: 1.44 to 3.10) higher rate of RRT initiation than those in the lowest quartile. Similarly, higher time-average eosinophil quartiles were associated with an increased rate of RRT initiation (Table 3). The clinical characteristics according to time-average eosinophil quartiles are summarized in Table S1.
Table 3
Association between eosinophil quartiles and clinical outcomes

<table>
<thead>
<tr>
<th>Outcome: Eosinophil quartiles</th>
<th>Baseline Cox model</th>
<th>Time-Average Cox model</th>
<th>Group-based trajectory model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q1: &lt; 90 (n = 688)</td>
<td>Q2: 90–170 (n = 684)</td>
<td>Q3: 170–289 (n = 736)</td>
</tr>
<tr>
<td>No. of events</td>
<td>71</td>
<td>79</td>
<td>139</td>
</tr>
<tr>
<td>Incidence rate, 100 p-y (95% CI)</td>
<td>1.4 (1.1–1.7)</td>
<td>1.5 (1.2–1.9)</td>
<td>2.7 (2.3–3.2)</td>
</tr>
<tr>
<td>Hazard Ratio (95% CI)</td>
<td>1.00 (ref)</td>
<td>1.18 (0.77–1.78)</td>
<td>1.77 (1.20–2.61)</td>
</tr>
<tr>
<td>P-value</td>
<td>-</td>
<td>0.43</td>
<td>0.004</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome: CV events and death</th>
<th>Baseline Cox model</th>
<th>Time-Average Cox model</th>
<th>Group-based trajectory model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q1: &lt; 90 (n = 688)</td>
<td>Q2: 90–170 (n = 684)</td>
<td>Q3: 170–289 (n = 736)</td>
</tr>
<tr>
<td>No. of events</td>
<td>87</td>
<td>89</td>
<td>104</td>
</tr>
<tr>
<td>Incidence rate, 100 p-y (95% CI)</td>
<td>1.7 (1.4–2.1)</td>
<td>1.7 (1.4–2.1)</td>
<td>2.0 (1.7–2.5)</td>
</tr>
<tr>
<td>Hazard Ratio (95% CI)</td>
<td>1.00 (ref)</td>
<td>0.94 (0.63–1.40)</td>
<td>1.17 (0.77–1.76)</td>
</tr>
<tr>
<td>P-value</td>
<td>-</td>
<td>0.8</td>
<td>0.47</td>
</tr>
</tbody>
</table>

The models were adjusted for age, sex, diabetes mellitus, body mass index, systolic blood pressure, chronic respiratory diseases, cardiovascular comorbidities, prior history of catheterization, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, loop diuretics, thiazide diuretics, mineralocorticoid receptor antagonists, proton pump inhibitors, histamine H2 receptor antagonists, non-steroidal anti-inflammatory drugs, hemoglobin, sodium, potassium, albumin, estimated glomerular filtration rate, C-reactive protein, urinary protein-to-creatinine ratio, and white blood cell count.

Abbreviations: RRT, renal replacement therapy; p-y, person-years; CI, confidence interval; CV, cardiovascular.

Group-based trajectory model
Three distinct trajectories of eosinophil counts were identified: low (n = 324), middle (n = 1,430), and high (n = 1,123) (Fig. 3). The clinical characteristics in each trajectory group are presented in Table S2. In a multivariable Cox model, patients in the high-trajectory group showed a 2.30-fold (95% CI: 1.38 to 3.84) higher rate of RRT initiation than those in the low-trajectory group (Table 3).

**Msm**

In MSM, high eosinophil counts (≥ 289 /µL) were associated with a 1.83-fold (95% CI: 1.33 to 2.51) higher rate of RRT initiation than normal eosinophil counts (Table 4). There was no significant effect modification by *a priori* defined baseline covariates, i.e., age, sex, BMI, systolic blood pressure, DM, cardiovascular comorbidities, hemoglobin, albumin, CKD stage, UPCR, and ACEI/ARB use. High eosinophil counts were significantly associated with a higher rate of RRT initiation when using the other definition of high eosinophil count (≥ 500 /µL).

**Table 4**
Marginal structural models for the association between high eosinophil counts and outcomes

<table>
<thead>
<tr>
<th>High eosinophil counts (/µL)</th>
<th>RRT initiation</th>
<th>CV events and mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>≥ 289 (vs. 289)</td>
<td>1.83 (1.33–2.51)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>≥ 500 (vs. &lt; 500)</td>
<td>1.41 (1.11–1.80)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

The models were adjusted for the baseline and time-dependent covariates. Baseline covariates included age, sex, body mass index, systolic blood pressure, diabetes mellitus, chronic respiratory diseases, cardiovascular comorbidities, prior history of catheterization, hemoglobin, albumin, estimated glomerular filtration rate, sodium, potassium, C-reactive protein, urinary protein-to-creatinine ratio, loop diuretics, thiazide diuretics, mineralocorticoid receptor antagonists, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, non-steroidal anti-inflammatory drugs, proton pump inhibitors, and histamine H₂ receptor antagonists. Time-dependent covariates included catheterization performed during follow-up, hemoglobin, albumin, estimated glomerular filtration rate, sodium, potassium, C-reactive protein, urinary protein-to-creatinine ratio, loop diuretics, thiazide diuretics, mineralocorticoid receptor antagonists, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, nonsteroidal anti-inflammatory drugs, proton pump inhibitors, histamine H₂ receptor antagonists, and corticosteroids.

**Cardiovascular events and mortality**

A total of 275 patients developed cardiovascular events (1.4 per 100 patient-years; 95% CI: 1.2 to 1.5) and 165 died (0.8 per 100 patient-years; 95% CI, 0.7 to 0.9). The association of eosinophil count with cardiovascular events and mortality was not significant in any statistical models except for MSM, where higher eosinophil counts were significantly associated with higher rates of these outcomes. (Tables 3 and 4).

**Sensitivity analysis**

After excluding patients with chronic respiratory diseases or cholesterol embolism, higher eosinophil counts were still associated with a higher rate of RRT initiation (Table S3). A similar result was obtained when including patients followed up for at least 90 days.

**Effects of oral carbon adsorbent, AST-120, on eosinophil counts**
This post-hoc analysis of the randomized controlled trial included 123 patients with stage 3–4 CKD (70 in the AST-120 group and 53 in the control group). The mean (SD) baseline eosinophil counts were 288 (442) /µL and 454 (1,364) /µL in the AST-120 and control groups, respectively. At 12 months, the mean (SD) eosinophil count was 340 (442) /µL and 456 (1,447) /µL in the AST-120 and control groups, respectively. A linear mixed-effects model for repeated measures showed no significant difference in the eosinophil counts between groups (P = 0.44) (Figure S2).

**Discussion**

We found a dose-dependent relationship between eosinophil counts and the risk of RRT initiation among patients with advanced CKD. The results were consistent when longitudinal alterations in eosinophil counts were modeled using the group-based trajectory modeling and MSM. Although future mechanistic studies are required to validate our findings, our data suggest the possible involvement of eosinophils in the progression of CKD.

Studies that examined the association between eosinophils and CKD progression are sparse\(^9\),\(^{15}\). These studies were limited by the small sample size, highly selective patient population, and insufficient adjustment for relevant confounders. More importantly, they did not consider longitudinal alterations in eosinophil counts despite the fact that eosinophils increase as kidney function declines\(^8\),\(^{9}\). We demonstrated a significant association between eosinophil counts and kidney outcomes in the group-based trajectory modeling and MSM that could capture longitudinal changes in eosinophil counts. Furthermore, MSM revealed that this association was independent of time-dependent confounding factors such as eGFR. Thus, although causality cannot be proven, our study provides plausible evidence regarding the link between eosinophils and CKD progression.

Several factors may confound the association between eosinophilia and CKD progression. First, chronic obstructive pulmonary disease and bronchial asthma were strongly associated with high eosinophil counts. They might accelerate CKD progression through hypoxia and inflammation\(^{31}–^{33}\), and thereby confound the association between eosinophilia and kidney outcomes. Second, drugs such as PPIs, diuretics, and NSAIDs induce both kidney injury and eosinophilia\(^{34}–^{37}\), and thus could be potential confounders. Finally, arterial catheterization sometimes causes cholesterol embolism, characterized by progressive kidney injury and eosinophilia\(^{38}\). Nevertheless, we did not find an association between catheterization and eosinophil count, most likely because cholesterol embolism is a very rare event. It should be emphasized that we demonstrated a significant association between eosinophil count and the kidney outcome after adjustment for these confounders.

One of the putative mechanisms linking eosinophils to CKD progression is their pro-atherogenic properties\(^5\). Since intrarenal arteriosclerosis/arteriolosclerosis leads to glomerulosclerosis and interstitial fibrosis and tubule atrophy (IFTA)\(^{39}–^{41}\), and is associated with worse renal prognosis\(^{42}\), eosinophils might affect kidney outcomes by accelerating nephrosclerotic lesions. Future studies are needed to clarify the effect of eosinophils on intrarenal arteriosclerosis/arteriolosclerosis.

Another possible explanation is that eosinophils may be involved in tubulointerstitial injury. Interstitial eosinophilic infiltration is typically observed in drug-induced tubulointerstitial nephritis, eosinophilic granulomatosis with polyangiitis\(^{12}\), tubulointerstitial nephritis with uveitis\(^{43}\), and idiopathic hypereosinophilic syndrome\(^{10},^{11}\). Interestingly, interstitial eosinophilic aggregates are also found in common kidney diseases, and are associated with IFTA, interstitial edema, and eosinophilic tubulitis, suggesting that they may aggravate tubulointerstitial inflammation and fibrosis\(^{13},^{14}\). The clinical implications of infiltrating eosinophils in the renal interstitium in terms of renal prognosis requires further detailed investigation.
A novel perspective has been proposed that eosinophils exert a tissue-protective effect. Liu et al. reported a cardioprotective role of interleukin-4 and mEar1 (human ECP ortholog) produced by eosinophils in a mouse model of myocardial infarction, suggesting that an increase in eosinophils in the heart and blood after myocardial infarction represents a compensatory protective response\textsuperscript{44}. Similarly, eosinophils prevent transverse aortic constriction-induced cardiac hypertrophy by inhibiting cardiomyocyte apoptosis and cardiac fibrosis\textsuperscript{45}. A protective role of eosinophils against liver injury has also been reported\textsuperscript{46}. Thus, higher eosinophil counts related to an increased risk of CKD progression may indicate a protective response to a more active disease status. However, these studies evaluated the effect of eosinophil deficiency, which may not be extrapolated to that of eosinophilia. Moreover, whether eosinophils play a protective or harmful role may depend on the target organs and the specific pathological context. The precise role of eosinophils in the progression of CKD requires further investigation.

Although the exact mechanisms remain elusive as to why eosinophils increase in advanced CKD, evidence has implied a role of uremic toxins. Interleukin-5, a master cytokine for eosinophil development, is elevated in nephrectomized mice\textsuperscript{47}. Uremic toxins, such as indoxyl sulfate and p-cresol, upregulate intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in endothelial cells\textsuperscript{48,49}, which promote eosinophil migration and degranulation\textsuperscript{50,51}. Thus, these studies support the involvement of uremic toxins in eosinophil proliferation and activation. Nevertheless, we did not find a significant change in eosinophil counts after AST-120 administration in CKD patients. Although this neutral result may be due to insufficient removal of uremic toxins by the drug, it might indicate that the clinical impact of uremic toxins on eosinophils is trivial.

Several factors were related to higher eosinophil counts in our study. Eosinophilia is a well-known side effect of ACEIs, and we confirmed that ACEIs/ARBs users showed higher eosinophil counts. Male had higher eosinophil counts than female. This sex difference has also been observed in healthy individuals\textsuperscript{52}. Estrogen inhibits eosinophil production in the bone marrow and induces eosinophil apoptosis\textsuperscript{53}, which might explain higher eosinophil counts in men. The positive association between BMI and eosinophil counts may be explained by this sex difference, while there seems to be a complex relationship between body weight and eosinophils\textsuperscript{54}. In our study, the association between eosinophil count and kidney outcome was independent of sex and BMI. In addition, there was no significant effect modification by gender or BMI on this association.

Notably, even modest elevations in eosinophil counts were associated with CKD progression. This is consistent with previous studies showing that a modest increase in eosinophil count, below the definition of eosinophilia (≥ 500/µL)\textsuperscript{30}, is associated with atherosclerotic plaques\textsuperscript{55} and albuminuria\textsuperscript{56}. Therefore, physicians should be aware of the clinical implications of subclinical eosinophilia, especially among patients with CKD.

Higher eosinophil counts were also associated with an increased risk of cardiovascular events and mortality in MSM. However, this association was not confirmed in the other statistical models. This discrepancy may be because only MSM could capture time-series changes in eosinophil counts throughout the study period. Conversely, statistical models other than MSM used eosinophil counts only at baseline or during the first 12 months, and thereby may have introduced a misclassification bias. Additionally, most cardiovascular events in our study were hospitalizations for heart failure. Given that eosinophils contribute to atherosclerotic lesions\textsuperscript{57}, the predominance of non-atherosclerotic cardiac events would have compromised the sensitivity to detect the impact of eosinophils. Indeed, a previous cohort study reported that eosinophils are not associated with incident heart failure in the general population\textsuperscript{58}. Further exploration of the association between eosinophils and cardiac events in patients with CKD is required.

The strengths of this study include large sample size, long-term follow-up period, and abundant data on eosinophil counts repeatedly measured within individuals. Despite the retrospective study design, missing data on eosinophil counts...
were < 1%. We assessed clinically-meaningful hard outcomes. A variety of covariates potentially related to eosinophilia were adjusted in MSM.

Our study had several limitations. First, the observational study design precludes causal inferences between eosinophil and renal prognosis. Second, some patients had a small number of measurements for eosinophils, which might have reduced the accuracy of the exposure. Third, since we enrolled advanced CKD patients in Japan, the generalizability of our findings to patients with more preserved kidney function or other ethnic groups is unknown. Finally, because we did not have data on tissue eosinophils, we could not directly link blood eosinophil counts with kidney eosinophils. However, previous studies showed a correlation between blood and kidney eosinophil counts\(^{14,15}\).

In conclusion, higher eosinophil counts were associated with an increased risk of RRT initiation in CKD patients. This association was robust after adjusting for time-dependent confounders. Even a modest increase in eosinophil count was associated with poorer kidney outcomes. Our findings highlight the possible involvement of eosinophils in the pathogenesis of CKD, which has largely been ignored in this field. Further mechanistic studies are required to elucidate the exact role of eosinophils and their potential as therapeutic targets for CKD.

**Declarations**

**Acknowledgements**

Nothing to disclose

**Author Contributions**

KH and YS conceptualized the study, were responsible for methodology, data curation, formal analysis, validation, and visualization, and wrote the original draft; TO, TK, SK, and YA were responsible for data collection and revision of the draft; JYK, IM, MM and YI were responsible for supervision and revision of the draft.

**Data Availability Statement**

The data underlying this article cannot be shared publicly due to the privacy of individuals that participated in the study. The data will be shared on reasonable request to the corresponding author.

**Funding**

Nothing to disclose

**Conflict of interest statement**

Nothing to disclose

**References**


Figures
Figure 1

Statistical models used in this study

(A) Baseline Cox proportional hazards model: Association between baseline eosinophil counts and outcomes is analyzed.

(B) Time-average Cox proportional hazards model: Association between average eosinophil counts over the first 12 months and subsequent risk of outcomes is analyzed.

(C) Group-based trajectory model: Eosinophil count trajectories during the first 12 months of follow-up are identified by group-based trajectory modeling. Association between the trajectories and subsequent risk of outcomes is analyzed.

(D) Marginal structural model: Association between time-varying eosinophil counts and outcomes is analyzed with adjustment for baseline and time-dependent confounders.
Figure 2

A restricted cubic spline curve for the association between eosinophil counts and kidney function

The dashed lines indicate 95% confidence intervals. Three knots (10th, 50th, and 90th percentiles of eGFR) were used in restricted cubic spline regression. The bar graph shows the histogram of the study patients according to eGFR.

Abbreviations: eGFR, estimated glomerular filtration rate.
Eosinophil count trajectories identified by group-based trajectory models

Group-based trajectory modeling identified three distinct trajectories of eosinophil counts during the first 12 months of the follow-up period: high, middle, and low. The solid lines and dots represent the averaged estimated trajectory and averaged observed trajectory, respectively. The dashed lines indicate 95% confidence intervals.

Supplementary Files

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- EOSsupplementalSR.docx