Supplemental Material

Supplemental Methods

1) Demographics and clinical variables collected in the retrospective CKD cohort

Demographics and comorbidities were extracted from a chart review of patients' electronic medical records by nephrologists. These included age, sex, body mass index, blood pressure, diabetes mellitus, 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 the electronic data capture system developed at Osaka University Hospital. These included serum albumin, creatinine, sodium, potassium, C-reactive protein, hemoglobin, white blood cell counts, and 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 type-2 receptor antagonists, and corticosteroids.

The time-series data were collected at a monthly interval during the study period. If multiple data existed within a month, the datum taken on the day closest to each of the one-month time points was adopted.

We collected information regarding arterial catheterization performed during follow-up (cardiac catheterization and endovascular treatment for peripheral artery diseases and carotid artery stenosis) since these procedures may have contributed to the development of eosinophilia via cholesterol embolism. These data were obtained from diagnostic procedure combination (DPC) codes. DPC codes are used for inpatient hospital payment systems in Japan, which provide the main diagnosis and type of intervention (1).

Reference

(1) Hayashida K, Murakami G, Matsuda S, Fushimi K. History and Profile of Diagnosis Procedure Combination (DPC): Development of a Real Data Collection System for Acute Inpatient Care in Japan. J Epidemiol. 2021;31(1):1-11.

2) LASSO

LASSO is a regularization method that reduces the model overfitting and improves the predictive accuracy through shrinking coefficients of weaker predictors toward zero. To determine the tuning parameter λ that minimizes the out-of-sample mean squared error of the predictions produced by selected variables, we used an adaptive method, which is a refined iteration of cross-validation.

Based on clinical relevance, the following 19 variables were included in the model: age, sex, systolic blood pressure, body mass index, diabetes mellitus, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, loop diuretics, thiazide diuretics, mineralocorticoid receptor antagonists, proton pump inhibitors, histamine type-2 receptor antagonists, antibiotics, corticosteroids, nonsteroidal anti-inflammatory drugs, eGFR, urinary protein-to-creatinine ratio, blood eosinophil counts, and the histological chronicity score. All variables were standardized to a mean of zero and a standard deviation of

one. Odds ratios and confidence intervals were estimated using the cross-fit partialing-out method (STATA command "xpologit").

3) Statistical models used in the retrospective CKD cohort

Baseline Cox model

The association between baseline blood eosinophil quartiles and kidney outcome was analyzed using multivariable Cox proportional hazards models. The following baseline covariates were included in this model: age, sex, body mass index, systolic blood pressure, diabetes mellitus, cardiovascular comorbidities, chronic respiratory diseases, a prior history of arterial catheterization and cholesterol embolism, hemoglobin, albumin, eGFR, sodium, potassium, C-reactive protein, white blood cell counts, 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 type-2 receptor antagonists. Proportional hazards assumptions were checked based on complementary log-log plots and scaled Schoenfeld residuals.

Time-average Cox model

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

Group-based trajectory model

Group-based trajectory model was used to assess the association between blood eosinophil count trajectories during the first 12 months and subsequent rates of the outcome (STATA command, *traj*). All available data on blood eosinophil counts during the first 12 months were used to identify eosinophil count trajectories. In this analysis, blood 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 (1-4). Potential trajectory groups were estimated from individual longitudinal eosinophil data, using the maximum likelihood estimation method based on the finite mixture model theorem. 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, with at least 5% of all patients being in the smallest group.

After deriving the eosinophil trajectory groups, multivariable 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 Cox model. In this model, the onset of survival time was set at 12 months.

Marginal structural model

Marginal structural models were employed to 1) assess the time-varying blood eosinophil counts throughout the study period and 2) deal with time-dependent confounding between blood eosinophil counts and eGFR.

Marginal structural model is a statistical method that can account for time-dependent confounding (5-8). In the current study, eGFR was considered to be the main time-dependent confounder because it influenced both exposure (blood eosinophil counts) and kidney outcomes, while being possibly affected by previous blood 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 blood eosinophil counts were adopted: 1) blood eosinophil count ≥289/μL (the top 25th percentile in our cohort) and 2) blood eosinophil count ≥ 500/μL (9). 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 Cox model. Time-dependent covariates included arterial catheterization performed during follow-up, hemoglobin, albumin, eGFR, 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 type-2 receptor antagonists, and corticosteroids.

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

Multiple imputation

In the retrospective CKD cohort, missing data at baseline were imputed using the multiple imputations by chained equation method based on all baseline covariates. Since all missing data were continuous variables (body mass index, systolic blood pressure, eGFR, hemoglobin, sodium, potassium, urinary protein-to-creatinine ratio, albumin, and C-reactive protein), the data were imputed using linear regression imputation. We created ten imputed datasets that were analyzed separately and combined based on Rubin’s rule.

References

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4) Mori M, Krumholz HM, Allore HG: Using Latent Class Analysis to Identify Hidden Clinical Phenotypes. JAMA 324: 700-701, 2020
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6) Xie D, Yang W, Jepson C, Roy J, Hsu JY, Shou H, et al: Statistical Methods for Modeling Time-Updated Exposures in Cohort Studies of Chronic Kidney Disease. Clin J Am Soc Nephrol 12: 1892-1899, 2017
7) Lukowsky LR, Mehrotra R, Kheifets L, Arah OA, Nissenson AR, Kalantar-Zadeh K: Comparing mortality of peritoneal and hemodialysis patients in the first 2 years of dialysis therapy: a marginal structural model analysis. Clin J Am Soc Nephrol 8: 619-628, 2013
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9) Shomali W, Gotlib J: World Health Organization-defined eosinophilic disorders: 2022 update on diagnosis, risk stratification, and management. Am J Hematol 97:129-148, 2022

Supplemental Table 1. Clinicopathological diagnosis in the kidney biopsy cohort

Clinicopathological diagnosis (n=563)	n (%)	Interstitial eosinophilic aggregates n (%)
IgA nephropathy, n (%)	219 (39)	20 (9)
Minimal change disease, n (%)	73 (13)	6 (8)
Membranous nephropathy, n (%)	44 (8)	8 (18)
Nephrosclerosis, n (%)	33 (6)	7 (21)
Tubulointerstitial nephritis, n (%)	32 (6)	11 (34)
ANCA-associated glomerulonephritis, n (%)	30 (5)	5 (17)
Lupus nephritis, n (%)	25 (5)	4 (16)
Diabetic nephropathy, n (%)	22 (4)	11 (50)
Focal segmental glomerulonephritis, n (%)	17 (3)	5 (29)
Membranoproliferative glomerulonephritis, n (%)	8 (1)	4 (50)
Amyloid nephropathy, n (%)	7 (1)	1 (14)
Thrombotic microangiopathy, n (%)	5 (1)	2 (40)
Thin basement membrane disease, n (%)	5 (1)	1 (20)
Fabry disease, n (%)	4 (1)	0
Acute proliferative nephritis, n (%)	4 (1)	1 (25)
Endocapillary proliferative glomerulonephritis, n (%)	4 (1)	1 (25)
IgG4-related kidney disease, n (%)	3 (0.5)	1 (33)
Obesity-related glomerulopathy, n (%)	3 (0.5)	0
Alport syndrome, n (%)	2 (0.4)	0
Light chain deposition disease, n (%)	2 (0.4)	1 (50)
Preeclampsia, n (%)	2 (0.4)	0
Miscellaneous, n (%)	19 (5.6)	7 (37)

Abbreviation: ANCA; anti-neutrophil cytoplasmic antibody

Supplemental Table 2. Sensitivity analyses for the association between interstitial eosinophilic aggregates and kidney outcome

Models	Adjusted HR* (95% CI)	P-value
(1) Adding diabetes mellitus to the clinical/histological model**	3.47 (2.36-5.09)	<0.001
(2) Adding the interstitial cell infiltration score to the clinical/histological model**	3.99 (2.71-5.87)	<0.001
(3) Excluding the etiologies that contained <10 patients, tubulointerstitial nephritis, ANCA-associated nephropathy, and lupus nephritis (n=408)	3.50 (2.16-5.70)	<0.001

*Adjusted HR for the kidney outcome among patients with interstitial eosinophilic aggregates compared to those without interstitial eosinophilic aggregates.

**The clinical/histological model includes age, sex, eGFR, UPCR, and the histological chronicity score.

Abbreviations: eGFR, estimated glomerular filtration rate; UPCR, urinary protein-to-creatinine ratio; ANCA; anti-neutrophil cytoplasmic antibody; HR, hazard ratio; CI, confidence interval.

Supplemental Table 3. Baseline characteristics according to blood eosinophil quartile in the retrospective CKD cohort

			Blood eosinophil quartiles: range (/μL)			
	Total	Missing data	Q1: < 90	Q2: 90-170	Q3: 170-289	Q4: > 289
Characteristics	n=2,877	n (%)	n=688	n=684	n=736	n=769
Age, year	63(14)	0	63(14)	64(14)	63(14)	63(16)
Male, n (%)	1,873(65)	0	356(52)	435(64)	509(69)	573(75)
Diabetes mellitus, n (%)	1,173(41)	0	243(35)	264(39)	331(45)	335(44)
BMI, kg/m^2	23(4)	84 (3)	22(4)	23(4)	23(4)	24(4)
SBP, mmHg	131(21)	108 (4)	130(21)	132(20)	131(20)	131(21)
Cardiovascular comorbidities, n (%)	512(18)	0	78(11)	118(17)	136(18)	180(23)
Prior history of catheterization	, n (%)415(14)	0	71(10)	89(13)	106(14)	149(19)
Chronic respiratory diseases, n (%)	43(1)	0	7(1)	6(1)	9(1)	21(3)
ACEIs/ARBs, n (%)	485(17)	0	83(12)	103(15)	124(17)	175(23)
Loop diuretics, n (%)	299(10)	0	48(7)	63(9)	68(9)	120(16)
Thiazide diuretics, n (%)	101(4)	0	11(2)	24(4)	30(4)	36(5)
MRAs, n (%)	176(6)	0	29(4)	41(6)	39(5)	67(9)
NSAIDs, n (%)	63(2)	0	16(2)	12(2)	24(3)	11(1)
Proton pump inhibitors, n (%)	276(10)	0	52(8)	56(8)	61(8)	107(14)
H_2 blockers, n (%)	285(10)	0	68(10)	72(11)	73(10)	72(9)
Hemoglobin, g/dL	12.3(2.1)	1 (<0.1)	12.1(2.0)	12.5(2.0)	12.5(2.1)	12.2(2.1)
Sodium, mEq/L	140(3)	466 (16)	140(3)	140(3)	140(3)	139(3)
Potassium, mEq/L	4.4(0.5)	356 (12)	4.4(0.6)	4.4(0.5)	4.5(0.5)	4.5(0.5)
Albumin, g/dL	3.8(0.6)	695 (24)	3.8(0.6)	3.9(0.6)	3.8(0.6)	3.7(0.6)

eGFR, ml/min/1.73m^2	37(14)	50 (3)	39(14)	38(14)	37(14)	35(14)
C-reactive protein, mg/dL	0.1[0.0-0.9]	760 (26)	0.1[0.0-0.7]	0.1[0.0-0.7]	0.1[0.0-1.0]	0.2[0.0-1.1]
UPCR, g/gCre	0.7[0.0-2.4]	1070 (37)	0.4[0.0-1.8]	0.6[0.0-2.2]	0.8[0.0-2.5]	0.9[0.0-2.7]
White blood cells, ×10^3/μL	7.2(3.4)	0	6.7(3.5)	6.4(2.2)	7.3(3.5)	8.3(3.7)

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

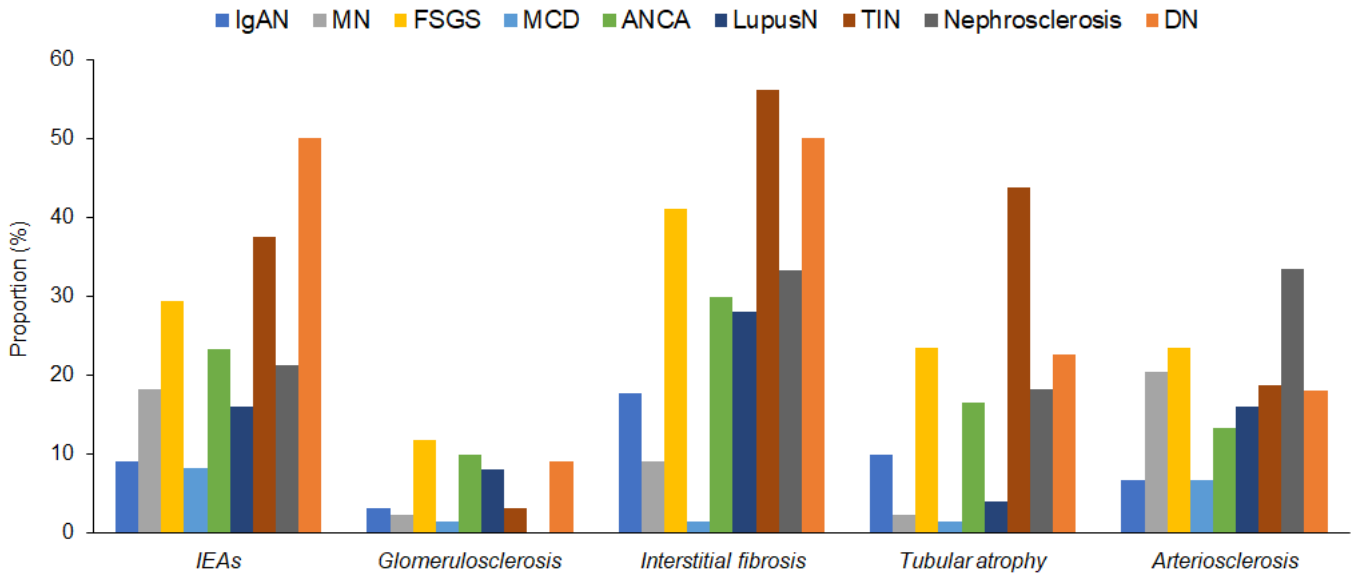
Abbreviations: BMI, body mass index; SBP, systolic blood pressure; ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; MRAs, mineralocorticoid receptor antagonists; NSAIDs, non-steroidal anti-inflammatory drugs; H_2 blockers, histamine H_2 receptor antagonists; eGFR, estimated glomerular filtration rate; UPCR, urinary protein-to-creatinine ratio

Supplemental Table 4. Adjusted hazard ratios for kidney outcome in the baseline Cox proportional hazards model

Variables	Adjusted HR [95% CI]	P-value
eGFR, per 10 ml/min/1.73 m^2 lower	1.61 [1.41-1.85]	<0.001
UPCR, per 1 g/gCre higher	1.36 [1.06-1.74]	0.02

Abbreviations: HR, hazard ratio; CI, confidence interval; eGFR, estimated glomerular filtration rat; UPCR, urinary protein-to-creatinine ratio.

Supplemental Figure 1. Proportion of patients with interstitial eosinophilic aggregates and each component of the histological chronicity score (≥score 2) across etiologies

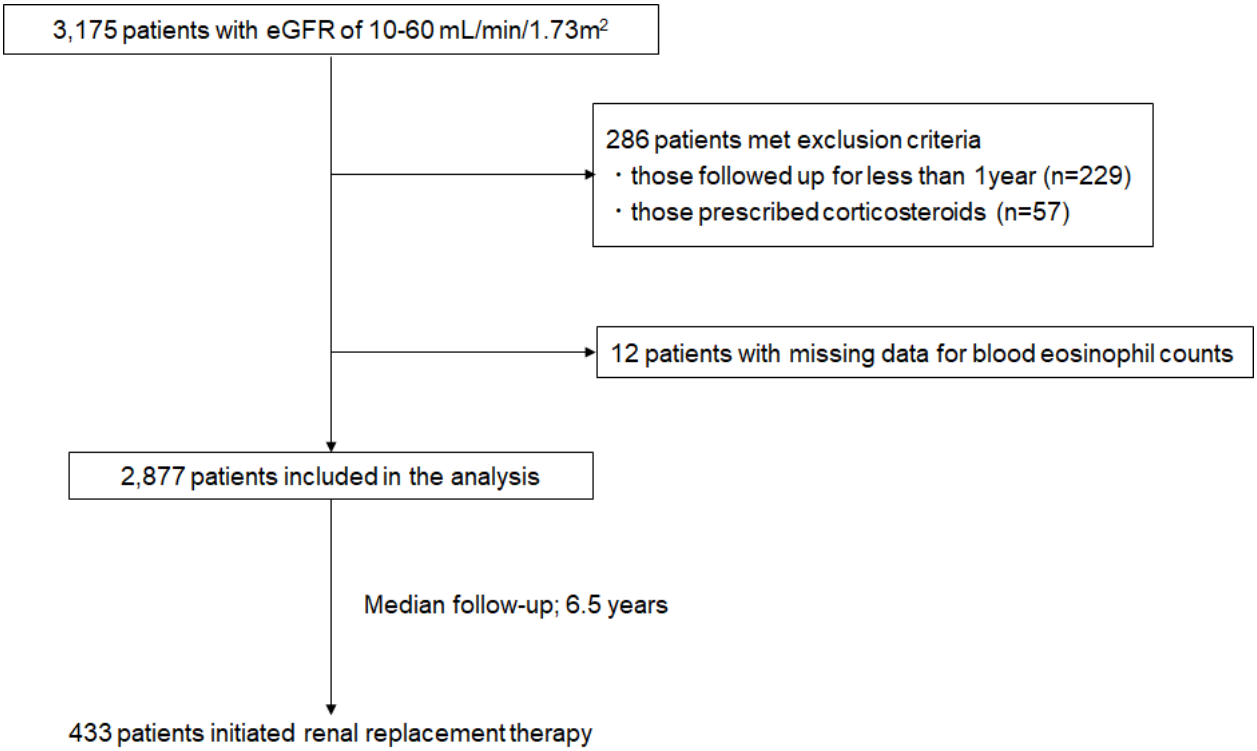


While interstitial eosinophilic aggregates are most frequently observed in DN (50%), note that they are detected across a wide range of etiologies.

The proportions of patients with the histological scores (glomerulosclerosis, interstitial fibrosis, tubular atrophy, and arteriosclerosis) ≥2 are presented.

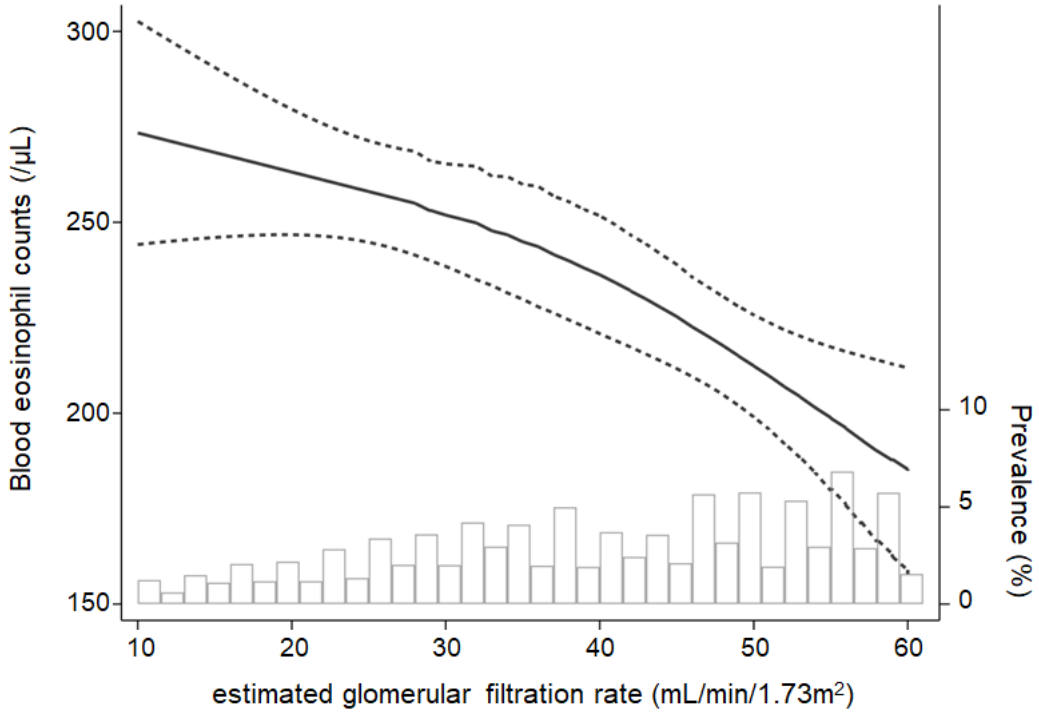
Abbreviations: IgAN, IgA nephropathy; MN, membranous nephropathy; FSGS, focal and segmental glomerulosclerosis; MCD, minimal change disease; ANCA, anti-neutrophil cytoplasmic antibody-associated glomerulonephritis; LupusN, Lupus nephritis; TIN Tubulointerstitial nephritis; DN, diabetic nephropathy

Supplemental Figure 2. Flow diagram of the retrospective CKD cohort



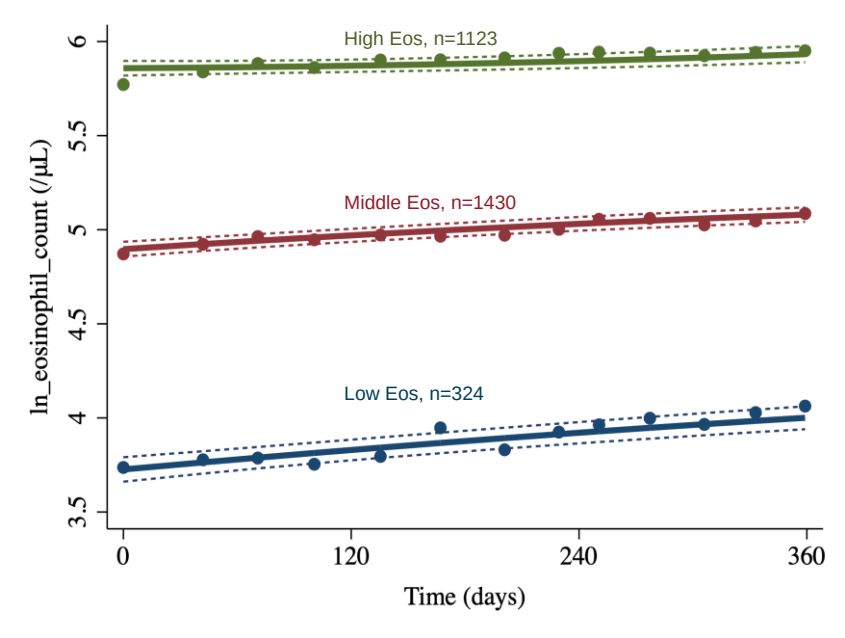
Abbreviation: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate

Supplemental Figure 3. A restricted cubic spline curve for the association between blood eosinophil counts and kidney function



Three knots (10th, 50th, and 90th percentiles of estimated glomerular filtration rate) are used in the restricted cubic spline regression. Dashed lines indicate 95% confidence intervals. Bar graph shows the histogram of the study patients according to estimated glomerular filtration rate.

Supplemental Figure 4. Blood eosinophil count trajectories identified by a group-based trajectory model



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