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Serum citrullinated histone H3 as a biomarker of disease activity and renal outcome in microscopic polyangiitis and granulomatosis with polyangiitis

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Abstract

Background Citrullinated histone H3 (CitH3), a hallmark component of neutrophil extracellular traps, has emerged as a circulating indicator of pathological neutrophil activation. We evaluated the utility of serum CitH3 as a biomarker for disease activity and risk of end-stage kidney disease (ESKD) development in patients with microscopic polyangiitis (MPA) and granulomatosis with polyangiitis (GPA).

Methods A total of 65 patients with MPA and GPA were included. At diagnosis, serum CitH3 levels were measured and disease activity was assessed using the Birmingham Vasculitis Activity Score (BVAS). Correlation between serum CitH3 level and BVAS was assessed using Pearson's correlation coefficient. Discriminatory performance of serum CitH3 for severe disease (BVAS ≥ 20) and low disease activity states (BVAS ≤ 3) was evaluated using receiver operating characteristic curve analysis. The association between serum CitH3 level and ESKD risk was assessed using multivariable Cox regression analysis.

Results Serum CitH3 levels significantly correlated with BVAS ($r = 0.393$, $p = 0.001$). Moreover, serum CitH3 demonstrated excellent discriminatory performance (area under the curve [AUC] 0.905, 95% confidence interval [CI] 0.825–0.984, $p < 0.001$) for severe disease (BVAS ≥ 20), and acceptable discriminatory performance (AUC 0.703, 95% CI 0.558–0.848, $p = 0.013$) for low disease activity states (BVAS ≤ 3). During a mean follow-up of 38.3 ± 32.8 months, eight (12.3%) patients developed ESKD. After adjusting for age and serum creatinine, higher serum CitH3 levels were independently associated with an increased risk of ESKD (adjusted hazard ratio 1.181, 95% CI 1.033–1.352, $p = 0.015$).

Conclusion Serum CitH3 correlated with disease activity, demonstrated strong discriminatory performance for severe disease and acceptable discriminatory performance for low disease activity states, and was associated with an increased risk of ESKD. These findings support serum CitH3 as a potential biomarker for identifying severe disease and low disease activity states, and stratifying ESKD risk in MPA and GPA.

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Keywords Citrullinated histone H3, Microscopic polyangiitis, Granulomatosis with polyangiitis, Disease activity, end-stage kidney disease

Background

Antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis (AAV) is a group of necrotizing small-vessel vasculitides characterized by pauci-immune inflammation affecting multiple organs [1]. Microscopic polyangiitis (MPA) and granulomatosis with polyangiitis (GPA) represent two major subtypes that share substantial overlap in clinical manifestations, immunopathogenesis, and therapeutic approaches [1–3]. MPA and GPA commonly follow a relapsing– remitting clinical course; thus, accurate disease activity assessment and monitoring are important [4]. The Birmingham Vasculitis Activity Score (BVAS), a comprehensive instrument incorporating numerous organ-specific items, is a widely used tool for assessing disease activity in MPA and GPA [5]. However, its complexity and time-consuming nature often limit its feasibility for rapid assessment in routine clinical practice. Accordingly, there is a clear unmet need for easily measurable biomarkers that can reflect disease activity in a timely manner.

End-stage kidney disease (ESKD) is one of the most clinically significant complications of MPA and GPA and remains a major determinant of long-term morbidity and survival [6]. Although overall mortality in AAV has improved with modern immunosuppressive strategies, progression to irreversible renal failure continues to impose a substantial clinical burden [7]. Early identification of patients at high risk for ESKD would allow intensified monitoring and potentially earlier therapeutic intervention. Therefore, identification of biomarkers associated with the risk of ESKD development is crucial in patients with MPA and GPA.

Dysregulated neutrophil activation and neutrophil extracellular trap (NET) formation play a central role in the pathogenesis of AAV [8]. In AAV, ANCA-primed neutrophils undergo excessive activation through Fc receptor- and complement-mediated pathways, leading to NETosis and subsequent endothelial injury, thereby contributing to ongoing vascular inflammation and organ damage [8]. Citrullinated histone H3 (CitH3), generated through peptidylarginine deiminase-mediated histone modification during NETosis, represents a major component of NETs and has emerged as a circulating indicator of pathological neutrophil activation [9]. Collectively, these findings provide a strong biological rationale for investigating CitH3 as a mechanistically grounded biomarker that may capture NET-driven disease activity and future organ damage, including ESKD, in patients with MPA and GPA.

Therefore, we hypothesized that serum CitH3 levels may reflect disease activity in patients with MPA and GPA and may be associated with the risk of progression to ESKD. In this study, we measured serum CitH3 levels in patients with MPA and GPA, examined their cross-sectional correlation with BVAS, and evaluated the association between CitH3 levels and the risk of future development of ESKD. Through this approach, we aimed to explore the potential role of serum CitH3 as a disease activity biomarker and risk factor for ESKD progression in MPA and GPA.

Methods

Patients

From a single-center cohort of 327 patients with AAV, 65 patients with MPA and GPA who had stored serum samples available were included in this study (Fig. 1). The inclusion criteria of the AAV cohort were as follows: (i) newly diagnosed with AAV in the Division of Rheumatology, Department of Internal Medicine of this institute from November 2005 to December 2023; (ii) fulfillment of the algorithm for classifying AAV proposed by the European Medicine Agency in 2007 and the revised nomenclature of systemic vasculitides suggested by the Chapel Hill Consensus Conference in 2012 [10, 11]; (iii) fulfillment of the 2022 American College of Rheumatology and European Alliance of Associations for Rheumatology classification criteria for MPA, GPA, and eosinophilic granulomatosis with polyangiitis [12–14]; (iv) presence of full electronic medical records on clinical, laboratory, radiological, and histological data, including demographics, ANCA type, disease manifestations, AAV-specific indices (BVAS, the five-factor score [FFS], and the vasculitis damage index [VDI]), and treatment information from AAV diagnosis to the last visit; v) ANCA tests performed within 2 weeks before or after AAV diagnosis; vi) followed up for at least 6 months after AAV diagnosis; and vii) provided informed consent for the use of clinical data and stored blood samples for future studies at the time of cohort enrolment. The exclusion criteria were as follows: (i) patients who had serious medical conditions such as active malignancies, severe infectious diseases requiring hospital admission, and concomitant autoimmune diseases at the time of the diagnosis; (ii) patients who were exposed to medications or had concomitant diseases that may affect ANCA positivity; and (iii) patients who had received glucocorticoids and/or immunosuppressive drugs before AAV diagnosis, including cyclophosphamide, rituximab, azathioprine,

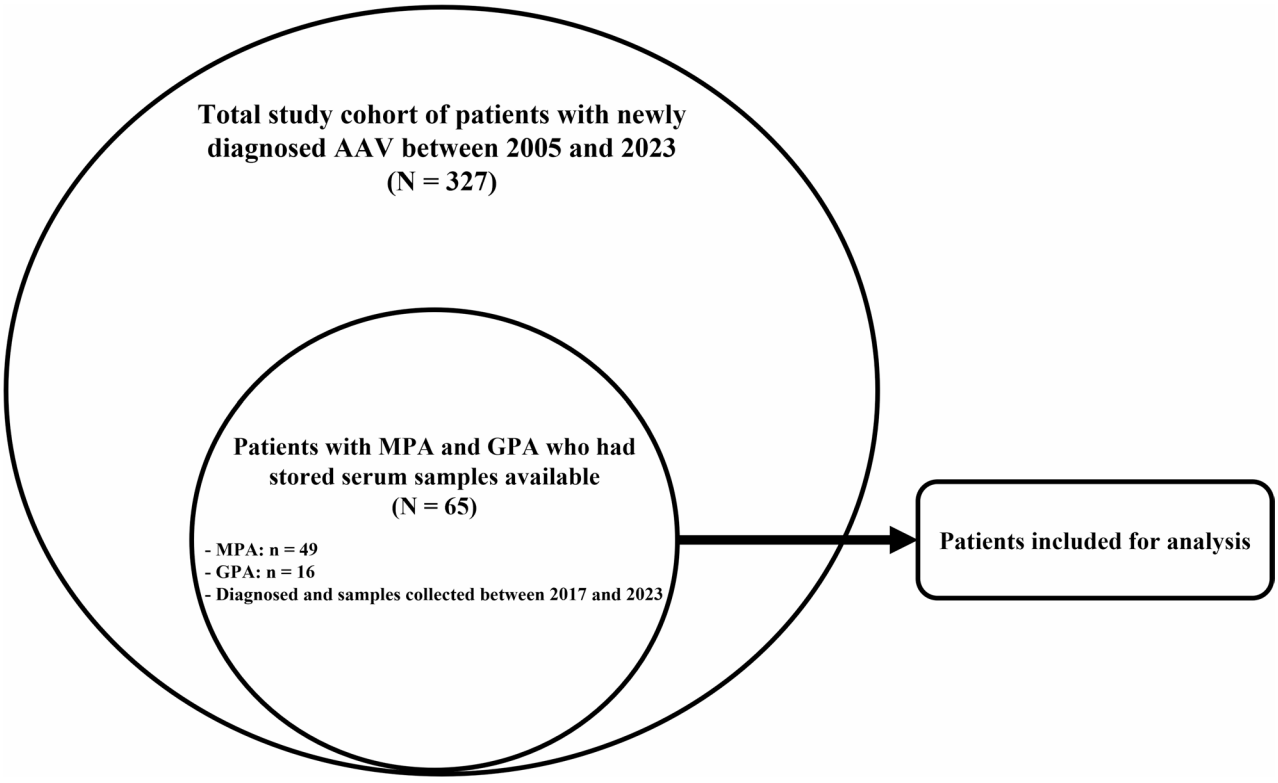

Fig. 1 Flow diagram of the selection of study population. *AAV* Antineutrophil cytoplasmic antibody–associated vasculitis, *MPA* Microscopic polyangiitis, *GPA* Granulomatosis with polyangiitis

mycophenolate mofetil, tacrolimus, cyclosporine, and methotrexate.

This study was approved by the Institutional Review Board (IRB) of Severance Hospital, Seoul, Republic of Korea (IRB number 4-2016-0901). All patients provided written informed consent upon enrolment in the cohort (at the time of AAV diagnosis and blood sampling).

Measurement of serum CitH3 levels

Whole blood was collected from the patients with their permission at the time of AAV diagnosis before administration of glucocorticoids and/or immunosuppressive drugs. The diagnosis and blood sample collection period for the included patients ranged from 2017 to 2023. On the same day, sera were subsequently isolated from whole blood and stored at -80 ℃. In our center, the time interval from blood collection to serum separation and freezing at -80 °C is typically within a few hours. Serum CitH3 was measured from the stored sera using enzyme-linked immunosorbent assay kits (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. There was no significant correlation between the year of diagnosis and serum CitH3 levels (r = -0.118, p = 0.349), suggesting that CitH3 levels were not influenced by the storage duration.

Covariates

The following data at baseline were included as covariates: demographics including age, sex, and smoking history (ex-smoker or not), AAV subtypes (MPA or GPA), ANCA positivity, AAV-specific indices (BVAS version 3 [5], FFS, and VDI), presence of comorbidities (type 2 diabetes, hypertension, and dyslipidemia), acute-phase reactants (erythrocyte sedimentation rate [ESR], and C-reactive protein [CRP]), laboratory data including white blood cell count, hemoglobin, platelet count, fasting glucose, total cholesterol, blood urea nitrogen, serum creatinine, total serum protein, and serum albumin levels.

The following medications were used during follow-up and were included as covariates: glucocorticoids, cyclophosphamide, rituximab, mycophenolate mofetil, azathioprine, tacrolimus, and methotrexate.

Outcome

The first outcome of our interest was the cross-sectional correlation between serum CitH3 levels and BVAS. Moreover, given that BVAS ≥ 20 is significantly associated with higher mortality and relapse rate than is BVAS < 20 [15, 16], the accuracy of CitH3 levels for discriminating BVAS ≥ 20 was also assessed as an outcome. In addition, considering the clinical relevance of identifying

low disease activity states on treatment decision, we further evaluated the discriminatory performance of serum CitH3 for low disease activity, defined as BVAS ≤ 3 [17].

The second outcome of our interest was the risk of ESKD development during follow-up. ESKD was defined as the initiation of long-term renal replacement therapy, including maintenance hemodialysis or peritoneal dialysis, or receipt of kidney transplantation. Time to ESKD was calculated from the date of AAV diagnosis to the date of ESKD development. Patients who did not develop ESKD were censored at the date of the last follow-up.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation and categorical variables as numbers (%). The correlation between serum CitH3 levels and BVAS was assessed using Pearson's correlation coefficient. The accuracy of serum CitH3 levels for discriminating BVAS ≥ 20 was assessed using the receiver operating characteristic (ROC) curve analysis. Area under the curve (AUC) and its 95% confidence interval (CI) were estimated. Cox proportional hazard regression analyses were performed to evaluate the association between serum CitH3 levels and risk of ESKD development. Death was treated as a censoring event; thus, patients who died contributed person-time from baseline to the time of death and were censored thereafter, consistent with a cause-specific Cox proportional hazard model. Univariable analysis was performed, followed by multivariable analysis. Factors with a $p < 0.05$ in the univariable analysis were included in the multivariable analysis, excluding those with multicollinearity (variance inflation factor [VIF] > 2.5; a relatively conservative threshold was applied, considering the relatively small sample size). To assess the robustness of the regression estimates and to account for the limited number of ESKD events, internal validation was performed using a bootstrapping approach with 1,000 iterations. Bootstrap estimates of regression coefficients, 95% CIs, and p values were obtained. In addition, to account for death as a competing risk, a sensitivity analysis was performed using a multivariable Fine–Gray subdistribution hazards model treating death as a competing risk.

Statistical significance was set at $p < 0.05$. All analyses were conducted using the SPSS software version 28.0 (IBM Corporation, Armonk, NY, USA).

Results

Patients' characteristics

The mean age of the 65 patients included in this study was 62.4 ± 14.4 years, and 37 (56.9%) patients were females. Forty-nine (75.4%) patients had MPA, and 16 (24.6%) had GPA. Myeloperoxidase-ANCA (or perinuclear-ANCA) was positive in 49 (75.4%) patients, and proteinase-3-ANCA (or cytoplasmic-ANCA) was positive in 14 (21.5%). The mean values of the BVAS, FFS, VDI were 10.6 ± 7.9, 1.5 ± 1.1, and 2.9 ± 1.9, respectively. The mean serum level of CitH3 was 8.9 ± 5.8 pg/mL. During a mean follow-up of 38.3 ± 32.8 months, 8 (12.3%) patients developed ESKD. Five (7.7%) patients died during follow-up. The detailed characteristics of the 65 patients are summarized in Table 1.

Serum CitH3 levels and disease activity

In Pearson's correlation analysis, serum CitH3 levels showed a significant positive correlation with BVAS ($r = 0.393$, $p = 0.001$) (Fig. 2). Moreover, serum CitH3 was highly accurate in discriminating BVAS ≥ 20, with an AUC of 0.905 (95% CI 0.825–0.984, $p < 0.001$) (Fig. 3A). Serum CitH3 was also significant in discriminating low disease activity states (BVAS ≤ 3), with an AUC of 0.703 (95% CI 0.558–0.848, $p = 0.013$) (Fig. 3B), indicating its utility as a biomarker for disease activity.

Serum CitH3 levels and risk of ESKD development

In the univariable Cox proportional hazard regression analysis, age (unadjusted hazard ratio [HR] 1.152, 95% CI 1.036–1.280, $p = 0.009$), hemoglobin level (unadjusted HR 0.598, 95% CI 0.394–0.906, $p = 0.015$), blood urea nitrogen level (unadjusted HR 1.040, 95% CI 1.017–1.063, $p < 0.001$), serum creatinine level (unadjusted HR 1.714, 95% CI 1.356–2.165, $p < 0.001$), serum albumin level (unadjusted HR 0.203, 95% CI 0.057–0.725, $p = 0.014$), and serum CitH3 level (unadjusted HR 1.127, 95% CI 1.008–1.261, $p = 0.037$) were significantly associated with the risk of ESKD development. After excluding hemoglobin (VIF 2.802), blood urea nitrogen (VIF 3.897), and serum albumin (VIF 2.541) owing to multicollinearity with serum creatinine, the multivariable analysis incorporated age, serum creatinine, and serum CitH3 into the model. Age (adjusted HR 1.154, 95% CI 1.027–1.297, $p = 0.016$), serum creatinine level (adjusted HR 2.197, 95% CI 1.411–3.420, $p < 0.001$), and serum CitH3 level (adjusted HR 1.181, 95% CI 1.033–1.352, $p = 0.015$) were significantly associated with a higher risk of ESKD development (Table 2).

In the bootstrap internal validation, the regression coefficients for age, serum creatinine level, and serum CitH3 level remained consistently positive, and their 95% CIs did not cross zero (Supplementary Table S1). Specifically, age (bootstrap estimate of B = 0.144, 95% CI 0.081–0.347, $p = 0.003$), serum creatinine (bootstrap estimate of B = 0.787, 95% CI 0.576–1.790, $p < 0.001$), and serum CitH3 (bootstrap estimate of B = 0.167, 95% CI 0.022–0.415, $p = 0.003$) all remained significantly associated with an increased risk of ESKD development.

Table 1 Characteristics of the 65 patients with MPA and GPA

Variables	Values
At diagnosis	
Demographic data	
Age, years, mean ± SD	62.4 ± 14.4
Male sex, n (%)	28 (43.1)
Female sex, n (%)	37 (56.9)
Ex-smoker, n (%)	2 (3.1)
AAV subtypes, n (%)	
MPA	49 (75.4)
GPA	16 (24.6)
ANCA positivity, n (%)	
MPO-ANCA (or P-ANCA) positive	49 (75.4)
PR3-ANCA (or C-ANCA) positive	14 (21.5)
Both ANCA positive	4 (6.2)
ANCA negative	6 (9.2)
AAV-specific indices	
BVAS, mean ± SD	10.6 ± 7.9
FFS, mean ± SD	1.5 ± 1.1
VDI, mean ± SD	2.9 ± 1.9
AAV manifestations, n (%)	
General	19 (29.2)
Cutaneous	6 (9.2)
Mucous and ocular	5 (7.7)
ENT	22 (33.8)
Pulmonary	37 (56.9)
Cardiovascular	6 (9.2)
Abdominal	0 (0.0)
Renal	38 (58.5)
Nervous system	14 (21.5)
Comorbidities, n (%)	
Type 2 diabetes mellitus	21 (32.3)
Hypertension	21 (32.3)
Dyslipidemia	11 (16.9)
Acute-phase reactants	
ESR, mm/hr, mean ± SD	57.6 ± 43.7
CRP, mg/L, mean ± SD	36.1 ± 51.3
Laboratory results, mean ± SD	
White blood cell count, /mm^3	9,433.8 ± 4,895.6
Hemoglobin, g/dL	11.5 ± 2.1
Platelet count, x 1,000/mm^3	309.7 ± 141.0
Fasting glucose, mg/dL	118.2 ± 54.7
Total cholesterol, mg/dL	172.1 ± 47.8
Blood urea nitrogen, mg/dL	24.7 ± 16.8
Serum creatinine, mg/dL	1.55 ± 1.54
Total serum protein, g/dL	6.8 ± 1.0
Serum albumin, g/dL	3.8 ± 0.6
Serum CitH3, pg/mL, mean ± SD	8.9 ± 5.8
During follow-up	
Medications, n (%)	
Glucocorticoids	60 (92.3)
Cyclophosphamide	36 (55.4)
Rituximab	16 (24.6)
Mycophenolate mofetil	30 (46.2)
Azathioprine	37 (56.9)

Table 1 (continued)

Variables	Values
Tacrolimus	5 (7.7)
Methotrexate	7 (10.8)
ESKD development, n (%)	8 (12.3)
Death during follow-up, n (%)	5 (7.7)
Follow-up duration, months, mean ± SD	38.3 ± 32.8

MPA Microscopic polyangiitis, *GPA* Granulomatosis with polyangiitis, *MPO* Myeloperoxidase, *ANCA* Antineutrophil cytoplasmic antibody, *P* Perinuclear, *C* Cytoplasmic, *PR3* Proteinase-3, *AAV* ANCA-associated vasculitis, *BVAS* Birmingham Vasculitis Activity Score, *FFS* Five-factor score, *VDI* Vasculitis damage index, *ENT* Ear, nose, and throat, *ESR* Erythrocyte sedimentation rate, *CRP* C-reactive protein, *CitH3* Citrullinated histone H3, *ESKD* End-stage kidney disease, *SD* Standard deviation

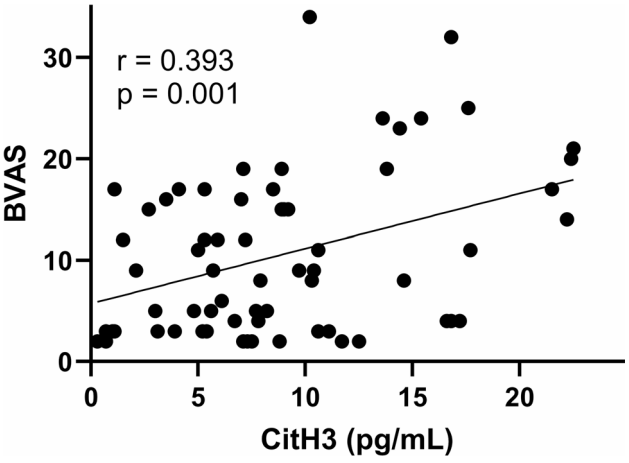

Fig. 2 Correlation between serum CitH3 levels and BVAS.*CitH3* Citrullinated histone H3, *BVAS* Birmingham Vasculitis Activity Score

Sensitivity analysis

In the sensitivity analysis using a multivariable Fine–Gray subdistribution hazards model accounting for death as a competing risk, higher serum CitH3 levels remained significantly associated with an increased risk of ESKD development (adjusted subdistribution HR 1.181, 95% CI 1.032–1.352, $p = 0.016$), after adjustment for age and serum creatinine (Table 3).

Discussion

In this study, we evaluated the clinical relevance of serum CitH3 in patients with MPA and GPA. Our key findings can be summarized as follows. First, serum CitH3 level was positively correlated with BVAS cross-sectionally, indicating that CitH3 could reflect contemporary disease activity. Notably, serum CitH3 demonstrated excellent discriminatory performance for the identification of severe disease defined as BVAS ≥ 20, and acceptable discriminatory performance for low disease activity states defined as BVAS ≤ 3. Second, higher serum CitH3 levels were independently associated with an increased risk of ESKD development during follow-up. Taken together, these findings suggest serum CitH3 as a potential biomarker that captures both disease activity and risk of ESKD development in patients with MPA and GPA.

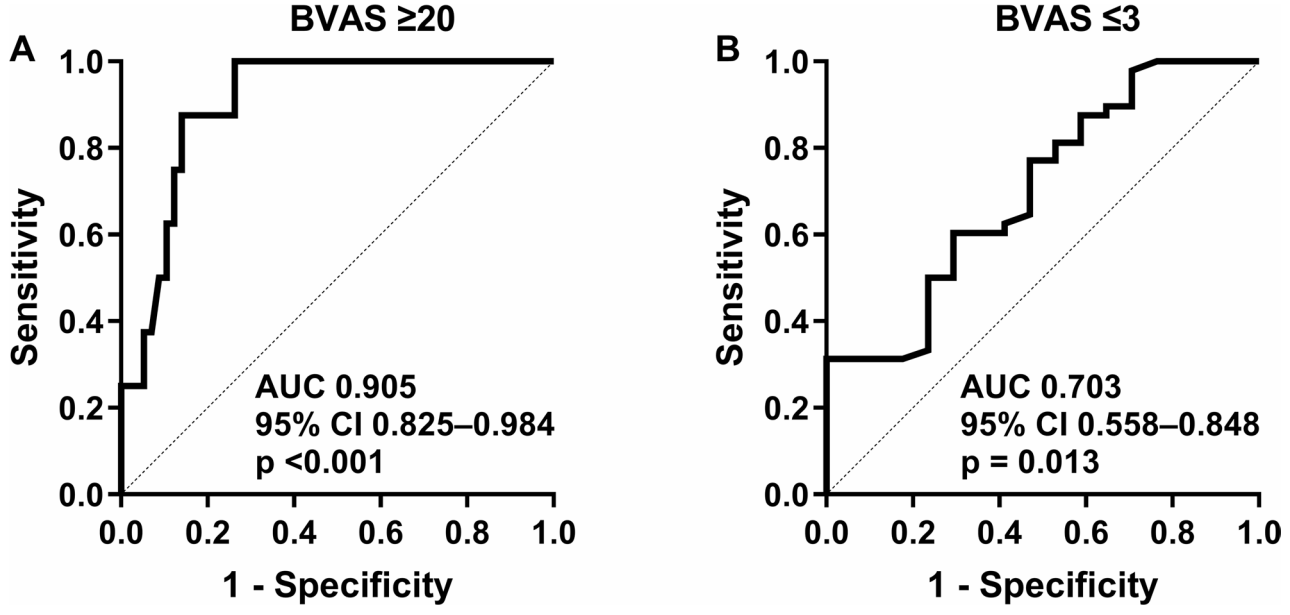

Fig. 3 Discriminatory performance of serum CitH3 for (**A**) severe disease (BVAS ≥ 20) and (**B**) low disease activity states (BVAS ≤ 3). *CitH3* Citrullinated histone H3, *BVAS* Birmingham Vasculitis Activity Score, *AUC* Area under the curve, *CI* Confidence interval

Table 2 Risk of ESKD development

Variables	Univariable		Multivariable	
	Unadjusted HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* value
Age, years	1.152 (1.036–1.280)	**0.009**	1.154 (1.027–1.297)	**0.016**
Male sex (vs. Female sex)	0.177 (0.022–1.438)	0.105		
Ex-smoker	0.047 (0.000–1,201,028.775)	0.726		
MPA (vs. GPA)	1.045 (0.211–5.182)	0.957		
MPO-ANCA (or P-ANCA) positive	2.574 (0.316–20.979)	0.377		
PR3-ANCA (or C-ANCA) positive	0.033 (0.000–38.407)	0.344		
BVAS	1.068 (0.992–1.150)	0.082		
FFS	1.879 (0.912–3.868)	0.087		
VDI	1.350 (0.980–1.859)	0.066		
Type 2 diabetes mellitus	0.295 (0.036–2.397)	0.253		
Hypertension	4.116 (0.978–17.333)	0.054		
Dyslipidemia	0.736 (0.091–5.981)	0.774		
ESR, mm/hr	0.996 (0.980–1.013)	0.661		
CRP, mg/L	1.001 (0.988–1.014)	0.878		
White blood cell count, /mm^3	1.028 (0.905–1.168)	0.668		
Hemoglobin, g/dL	0.598 (0.394–0.906)	**0.015**		
Platelet count, x1,000/mm^3	0.997 (0.990–1.003)	0.341		
Fasting glucose, mg/dL	1.000 (0.986–1.014)	0.953		
Total cholesterol, mg/dL	0.998 (0.982–1.013)	0.763		
Blood urea nitrogen, mg/dL	1.040 (1.017–1.063)	**< 0.001**		
Serum creatinine, mg/dL	1.714 (1.356–2.165)	**< 0.001**	2.197 (1.411–3.420)	**< 0.001**
Total serum protein, g/dL	0.531 (0.266–1.063)	0.074		
Serum albumin, g/dL	0.203 (0.057–0.725)	**0.014**		
Serum CitH3, pg/mL	1.127 (1.008–1.261)	**0.037**	1.181 (1.033–1.352)	**0.015**
Glucocorticoids	22.776 (0.000–1,896,839.897)	0.589		
Cyclophosphamide	1.281 (0.306–5.365)	0.735		
Rituximab	0.395 (0.048–3.213)	0.385		
Mycophenolate mofetil	0.359 (0.072–1.779)	0.210		
Azathioprine	0.403 (0.096–1.695)	0.215		
Tacrolimus	0.044 (0.000–3,656.732)	0.589		
Methotrexate	0.041 (0.000–605.869)	0.515		

ESKD End-stage kidney disease, *MPA* Microscopic polyangiitis, *GPA* Granulomatosis with polyangiitis, *MPO* Myeloperoxidase, *ANCA* Antineutrophil cytoplasmic antibody, *P* Perinuclear, *C* Cytoplasmic, *PR3* Proteinase-3, *BVAS* Birmingham Vasculitis Activity Score, *FFS* Five-factor score, *VDI* Vasculitis damage index, *ESR* Erythrocyte sedimentation rate, *CRP* C-reactive protein, *CitH3* Citrullinated histone H3, *HR* Hazard ratio, *CI* Confidence interval. *P* values < 0.05 are indicated in bold

Table 3 Sensitivity analysis: Multivariable Fine-Gray subdistribution hazard model

	Adjusted subdistribution HR (95% CI)	*P* value
Age, years	1.156 (1.028–1.299)	0.015
Serum creatinine, mg/dL	2.212 (1.420–3.446)	< 0.001
Serum CitH3, pg/mL	1.181 (1.032–1.352)	0.016

HR Hazard ratio, *CI* Confidence interval, *CitH3* Citrullinated histone H3

In routine practice, BVAS remains a reference standard for disease activity assessment [5]; however, practical constraints often limit timely and repeated scoring. Within this context, the significant correlation between CitH3 and BVAS corroborates the role of serum CitH3 as a convenient adjunct for disease activity assessment. Notably, the clinical utility of serum CitH3 was pronounced in discriminating severe disease (BVAS ≥ 20) with an AUC of 0.905 (95% CI 0.825–0.984). Accordingly, serum CitH3 may serve as a practical biomarker for early identification of patients with clinically significant high disease activity, thereby reinforcing clinical vigilance at the time of diagnosis. In addition, given the discriminatory ability of serum CitH3 for low disease activity states, it could also serve as a potential biomarker to identify patients who would still require immune-modulatory therapy.

The utility of serum CitH3 in assessing disease activity, particularly for identifying severe disease, can be explained by its close linkage to NET-driven pathogenic processes in AAV. Excessive neutrophil activation and NET formation are disproportionately amplified in severe disease states characterized by endothelial injury and organ-threatening inflammation [18, 19]. CitH3, generated during NETosis, reflects the intensity of this pathogenic neutrophil activity and thus captures a state

of enhanced NET-associated inflammatory burden that underlies clinically severe disease [20, 21]. Accordingly, elevated serum CitH3 levels likely indicate pathogenic intensification of NET-associated inflammation, providing a biological basis for its robust ability to distinguish patients with severe disease and supporting its clinical relevance as a biomarker of severe disease activity in MPA and GPA.

Another clinically important finding of our study is that serum CitH3 was an independent risk factor for ESKD development. Consistent with prior literature [22, 23], older age (adjusted HR 1.154, 95% CI 1.027–1.297, $p = 0.016$) and higher baseline serum creatinine level (adjusted HR 2.197, 95% CI 1.411–3.420, $p < 0.001$) were significantly associated with a higher risk of ESKD development, reaffirming that age and renal function at diagnosis remain key determinants of long-term renal outcome. Importantly, higher serum CitH3 level (adjusted HR 1.181, 95% CI 1.033–1.352, $p = 0.015$) remained significantly associated with a higher risk of ESKD development even after adjusting for age and serum creatinine levels. This suggests that CitH3 provides additional information beyond traditional clinical and laboratory parameters. From a clinical perspective, this finding supports the potential use of serum CitH3 for risk stratification at diagnosis regarding ESKD development, thus complementing established indicators.

We acknowledge that the number of ESKD events was small ($n = 8$), raising concerns regarding overfitting in the multivariable Cox regression analysis, which incorporated three covariates. To address this concern, we applied an internal validation approach using bootstrapping, which is commonly used to evaluate the robustness of regression estimates when event counts are low relative to model complexity [24]. The bootstrap results further supported the robustness of the observed association between serum CitH3 and the risk of ESKD development. Across 1,000 bootstrap resamples, the regression coefficient for CitH3 remained consistently positive, with a bootstrap estimate of B = 0.167 and a narrow 95% CI that did not cross zero (95% CI 0.022–0.415, $p = 0.003$) (Supplementary Table S1). Importantly, both the direction and magnitude of the association were preserved after resampling, indicating that the association between higher serum CitH3 levels and a higher risk of ESKD development was stable and not driven by a small number of events. Similar stability was also observed for age and serum creatinine, further supporting the internal coherence of the multivariable model. Collectively, these findings bolster confidence that the association between serum CitH3 and risk of ESKD development reflects a reproducible signal rather than random variation arising from the limited number of events.

A plausible explanation for the independent association between serum CitH3 and risk of ESKD development is the implication of NET-mediated injury in glomerular endothelial damage, capillary necrosis, and crescent formation, all of which contribute to irreversible renal damage in AAV [25]. CitH3 functions as a damage-associated molecular pattern that amplifies inflammatory responses and tissue injury, thereby linking NETosis to disease severity and adverse outcomes [26–28]. Experimental and translational studies further suggest that circulating CitH3 levels dynamically reflect inflammatory burden and treatment response, supporting its potential clinical relevance as a measurable indicator of organ involvement [9, 29, 30]. Elevated CitH3 at diagnosis may thus reflect an underlying state of sustained and dysregulated NET activity that not only manifests as severe systemic disease but also predisposes to progressive renal damage beyond what is captured by baseline serum creatinine alone. Importantly, the persistence of serum CitH3 as an independent risk factor after adjusting for age and baseline serum creatinine indicates that serum CitH3 may reflect additional pathogenic processes contributing to renal progression, potentially related to NET-mediated tissue injury, which is not fully reflected by conventional clinical parameters at diagnosis. Collectively, these considerations support the concept that serum CitH3 functions as a marker of pathogenic intensity and tissue-destructive potential, thereby explaining its particular utility in identifying patients with severe disease phenotypes and heightened risk of ESKD progression.

It is noteworthy that the rate of ESKD development in our study was similar to that in the CYCLOPS trial [31], despite better baseline kidney function in our study. This may be attributable to differences in study population characteristics, particularly geographic/ethnic background and disease phenotype distribution. The CYCLOPS trial was conducted predominantly in Western populations [31], whereas the present study included Korean patients. Notably, Asian cohorts have been reported to have a higher proportion of MPA compared with Western cohorts [32]. Consistent with this, the proportion of MPA was 47.7% in the CYCLOPS trial, whereas it was substantially higher in our cohort (75.4%). Because MPA is associated with a higher risk of ESKD progression compared with GPA [33, 34], the predominance of MPA in our cohort may have contributed to the observed ESKD rate.

Some limitations of this study should be acknowledged. First, as a single-center study with a relatively small sample size, the generalizability of the findings may be limited. Second, serum CitH3 was measured only at diagnosis, precluding assessment of longitudinal changes in relation to treatment response or relapse. Third, mechanistic inferences are indirect and require additional

experimental investigation. Further validation in larger cohorts, together with dedicated mechanistic studies, is required to establish the broader clinical applicability of serum CitH3.

Conclusions

In conclusion, serum CitH3 levels correlated with disease activity at diagnosis and exhibited strong discriminatory performance for severe disease and acceptable discriminatory performance for low disease activity states in patients with MPA and GPA. In addition, higher serum CitH3 levels were independently associated with an increased risk of ESKD development, even after adjusting for established risk factors. Collectively, these findings support serum CitH3 as a potential biomarker for detecting severe disease and low disease activity states, and stratifying the risk of ESKD development. Further studies with larger populations and longitudinal measurements are warranted to confirm and extend these observations.

Abbreviations

ANCA	Antineutrophil cytoplasmic antibody
AAV	Antineutrophil cytoplasmic antibody–associated vasculitis
MPA	Microscopic polyangiitis
GPA	Granulomatosis with polyangiitis
BVAS	Birmingham Vasculitis Activity Score
ESKD	End-stage kidney disease
NET	Neutrophil extracellular trap
CitH3	Citrullinated histone H3
FFS	Five-factor score
VDI	Vasculitis damage index
IRB	Institutional Review Board
ESR	Erythrocyte sedimentation rate
CRP	C-reactive protein
ROC	Receiver operating characteristic
AUC	Area under the curve
CI	Confidence interval
VIF	Variance inflation factor
HR	Hazard ratio

Supplementary Information

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Supplementary Material 1.

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None.

Authors' contributions

OCK contributed to study conception and design, analysis, interpretation of data, supervision, draft manuscript preparation, and revision of the manuscript for important intellectual content. TY contributed to study conception and design, analysis, interpretation of data, supervision, and revision of the manuscript for important intellectual content. JWH contributed to analysis and interpretation of data. Y-BP contributed to analysis and interpretation of data. S-WL contributed to study conception and design, interpretation of data, supervision, and revision of the manuscript for important intellectual content. S-WL is the guarantor and accepts full responsibility for the work and the conduct of the study, had access to the data, and controlled the decision to publish. All authors reviewed the results and approved the final version of the manuscript.

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Data availability

All data generated or analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board (IRB) of Severance Hospital, Seoul, Republic of Korea (IRB number 4-2016-0901). All patients provided written informed consent upon enrolment in the cohort (at the time of AAV diagnosis and blood sampling).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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