Serum Wnt3A levels are significantly associated with cross-sectional vasculitis activity and end-stage kidney disease during follow-up of patients with antineutrophil cytoplasmic antibody-associated vasculitis

T. Yoon[1], J.W. Ha[2], Y.-B. Park[3,4], S.-W. Lee[3,4]

[1]Department of Medical Science, BK21 Plus Project, Yonsei University, College of Medicine, Seoul; [2]Division of Rheumatology, Department of Internal Medicine, Yongin Severance Hospital, Yonsei University College of Medicine, Yongin, Gyeonggi-do; [3]Division of Rheumatology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul; [4]Institute for Immunology and Immunological Diseases, Yonsei University College of Medicine, Seoul, Republic of Korea.

Abstract

Objective

In this study, we investigated whether serum Wnt3A levels at diagnosis reflected cross-sectional activity and predicted poor outcomes during follow-up in patients with antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV).

Methods

This study included 80 patients who were newly diagnosed with AAV at a tertiary hospital. At diagnosis, whole blood was obtained from patients and sera was immediately isolated and stored at -80°C. Moreover, AAV activity was assessed using the Birmingham Vasculitis Activity Score (BVAS), and a high BVAS was defined as the highest tertile. Poor outcomes including all-cause mortality and end-stage kidney disease (ESKD) were recorded.

Results

The patients had a median age of 63.5 years, with 40% being male and 60% female patients. Serum levels of Wnt3A at diagnosis were correlated with the cross-sectional BVAS and serum Wnt3A ≥411.7 pg/mL exhibited an increased risk of high BVAS. In addition, serum Wnt3A levels at diagnosis significantly correlated with cross-sectional acute-phase reactants and serum albumin levels. Furthermore, serum Wnt3A levels at diagnosis were associated with AAV exacerbation, leading to ESKD. Particularly, serum Wnt3A ≥407.1 pg/mL also demonstrated an elevated risk of ESKD (relative risk 3.867). Additionally, patients with serum Wnt3A ≥407.1 pg/mL exhibited a significantly lower cumulative ESKD-free survival rate than those with lower serum Wnt3A levels.

Conclusion

This study is the first to demonstrate the clinical potential of serum Wnt3A levels at diagnosis for estimating cross-sectional activity and partially predicting the advancement to ESKD during follow-up in patients with AAV.

Key words

Wnt3A activity, end-stage kidney disease, antineutrophil cytoplasmic antibody, vasculitis

*Taejun Yoon, PhD**
*Jang Woo Ha, MD**
Yong-Beom Park, MD, PhD
Sang-Won Lee, MD, PhD

**Contributed equally.*

Please address correspondence to:
Sang-Won Lee
Division of Rheumatology,
Department of Internal Medicine,
Severance Hospital, Yonsei University College of Medicine,
50-1 Yonsei-ro, Seodaemun-gu,
Seoul, 03722, Republic of Korea.
E-mail: sangwonlee@yuhs.ac

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Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) constitutes a form of small-vessel vasculitis characterised by fibrinoid necrotising inflammation with few or no immune deposits in capillaries, adjacent arterioles and venules, and occasionally, arteries (1, 2). Additionally, AAV is divided into three subtypes according to distinct clinical, laboratory, radiological, and histological findings: microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), and eosinophilic GPA (EGPA) (3-6). Although all subtypes of AAV may theoretically involve almost all major organs, each AAV subtype has typical clinical features: MPA often affects the lungs and kidneys, GPA frequently affects the upper and lower respiratory tracts, and EGPA predominantly manifests as allergic diseases and peripheral neuropathy (2, 4-6). The Birmingham Vasculitis Activity Score (BVAS) version 3 is widely used in clinical practice to assess AAV activity. The BVAS is composed of nine items for organ-based systemic manifestations and one item for other manifestations (7). In addition, various factors at diagnosis have been identified to predict poor outcomes in AAV, including both traditional and AAV-specific risk factors (8, 9). Additionally, to date, numerous efforts have been made to discover serum biomarkers that reflect cross-sectional activity as well as predict poor outcomes of AAV (10-12). The advantages of these serum biomarkers over previously documented indicators are that they are operator-independent, objective, rapid, reproducible, and race- and region-specific.

Wnt proteins, cysteine-rich glycoproteins encoded by 19 human *Wnt* genes, play important roles in embryonic development and adult tissue homeostasis (13-15). As protein ligands, Wnt proteins may participate in two distinct signalling pathways including canonical (Wnt/β-catenin pathway) and non-canonical (the Wnt–planar cell polarity and the Wnt-Ca^{2}+ pathway) pathways (15, 16). Based on the functions of Wnt proteins and their related Wnt signalling pathways, Wnt proteins can be divided into two subgroups: Wnt 1 and 5. The Wnt 1 subgroup is linked to the canonical pathway (17). Recently, in addition to its essential biological functions, the Wnt signalling pathway is increasingly recognised for its involvement in the pathogenesis of various autoimmune diseases and cancers (18, 19). Particularly, serum levels of Wnt3A among the diverse Wnt proteins are recognised as new biomarkers in several cancers (17, 20-22). On the other hand, a previous study reported that Wnt3A might have the ability to control tumour necrosis factors (TNF)-α-induced interleukin (IL)-6 secretion (23). Given that these two cytokines are pivotal mediators of inflammation in the pathogenesis of AAV (24, 25), it can be reasonably speculated that serum levels of Wnt3A at diagnosis may reflect cross-sectional activity and further predict poor outcomes of AAV during follow-up. However, to date, no studies have demonstrated the clinical significance of serum Wnt3A levels in patients with AAV. Hence, in the present study, we investigated whether serum levels of Wnt3A at diagnosis could reflect cross-sectional activity and predict poor outcomes during follow-up in patients with AAV.

Patients and methods

Patients

In this study, we randomly selected 80 patients with AAV from the Severance Hospital ANCA-associated VasculitidEs (SHAVE) cohort (a prospective observational cohort of AAV). The inclusion criteria for the SHAVE cohort are briefly described as follows: i) the first diagnosis of AAV at the Division of Rheumatology, Department of Internal Medicine, the tertiary university hospital between 2000 and 2023; ii) the classification of AAV according to the 2007 European Medicines Agency algorithms for AAV and polyarteritis nodosa, as well as the 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides (1, 2); iii) the classification and/or reclassification of AAV according to the 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology clas-

sification criteria for AAV (4-6); iv) the presence of well-documented medical records sufficient to collect clinical data at diagnosis and during follow-up; v) the follow-up duration of at least 6 months or greater after diagnosis; vi) the absence of serious medical conditions mimicking AAV at diagnosis such as severe infectious and cancerous diseases (4-6); vii) no medical or drug history affecting ANCA positivity such as primary sclerosing cholangitis or propylthiouracil (26, 27); and viii) the absence of exposure to moderate to high doses of glucocorticoids or immunosuppressive drugs for AAV treatment within 4 weeks before diagnosis.

Ethical disclosure

This study was approved by the Institutional Review Board (IRB) of Severance Hospital, Seoul, Republic of Korea (IRB no.: 4-2016-0901). Written informed consent was obtained from all patients at the time of blood sampling. The IRB waived the need for written informed consent as it was previously obtained at entry into the SHAVE cohort.

Blood sampling

On the day of AAV diagnosis and assessment of AAV-specific indices, whole blood samples were obtained from patients with AAV. Sera was immediately isolated from whole blood and stored at -80°C.

Clinical and laboratory data

Regarding variables at diagnosis, the demographic data included age and sex. Moreover, AAV subtypes, ANCA type and positivity, systemic BVAS items, and AAV-specific indices were also assessed. AAV-specific indices include the BVAS, the Five-factor score (FFS), the 36-item short-form survey (SF-36), physical and mental component summaries (PCS and MCS), and vasculitis damage index (VDI) (7, 28-30). Laboratory results including acute-phase reactants, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels were also recorded. During follow-up, five poor outcomes were recorded after diagnosis, including all-cause mortality, relapse, end-stage kidney disease (ESKD), cerebrovascular accident (CVA), and acute coronary syndrome (ACS). For patients with a corresponding poor outcome, the follow-up duration was defined as the period from diagnosis to its occurrence, while for those without, it was defined as the duration from diagnosis to the last visit. The number of patients administered glucocorticoids and immunosuppressive drugs was also recorded.

Measurement of serum levels of Wnt3A

Serum levels of Wnt3A were measured from sera collected and stored at diagnosis using enzyme-linked immunosorbent assay kits (CusaBio, Houston, TX, USA).

Consensus of ANCA positivity

Titres of myeloperoxidase (MPO)-ANCA and proteinase 3 (PR3)-ANCA were measured using immunoassays, whereas perinuclear (P)-ANCA and cytoplasmic (C)-ANCA were detected using indirect immunofluorescence assays. In the present study, the results of MPO- and PR3-ANCAs, as well as P- and C-ANCAs, were used to determine ANCA positive (4-6).

High BVAS

In this study, high BVAS was arbitrarily defined as the highest tertile of BVAS (≥15) as per our previous studies (31, 32).

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics for Windows v. 26 (IBM Corp., Armonk, NY, USA). Continuous and categorical variables are expressed as medians (25~75 percentiles) and numbers (percentages). The correlation coefficient (r) between the two variables was obtained using Pearson correlation analysis. The significant area under the curve (AUC) was confirmed using receiver operator characteristic (ROC) curve analysis. The optimal cut-off was extrapolated by performing ROC curve analysis and selected as the value with the maximum sum of sensitivity and specificity. The relative risk (RR) of the cut-off for all-cause mortality was analysed using contingency tables and the chi-square test. The cumulative survival rates between the two groups were compared using Kaplan-Meier survival analysis with the log-rank test. The multivariate Cox hazard model using variables with statistical significance in the univariate Cox hazard model was used to obtain hazard ratios (HRs) during a considerable follow-up duration. Statistical significance was set at $p<0.05$.

Results

Characteristics of patients

In terms of variables at diagnosis, the median age of the 80 patients with AAV was 63.5 years, with 40% being male and 60% female patients. Of these 80 patients, 39, 24, and 17 were diagnosed with MPA, GPA, and EGPA, respectively. MPO-ANCA (or P-ANCA) and PR3-ANCA (or C-ANCA) positivity was observed in 45 and 12 patients, respectively. The median BVAS, FFS, SF-36 PCS, MCS, and VDI were 5.0. 0, 52.2, 55.3, and 3.0, respectively. The median ESR and CRP were 19.0 mm/h and 3.6 mg/L, respectively. The median serum creatinine and estimated glomerular filtration rate by the chronic kidney disease epidemiology collaboration (eGFR CKD-EPI) were 0.8 mg/dL and 92.2 mL/min/1.732m^2, respectively. Furthermore, the median serum levels of Wnt3A were 322.7 pg/mL. In terms of variables during follow-up, of the 80 patients, six died and 12 relapsed with AAV. Additionally, 19 patients progressed to ESKD, with four, and one experiencing CVA and ACS, respectively. Glucocorticoids were administered to 98.8% of the patients, with cyclophosphamide (66.3%) being the most frequently administered drug followed by azathioprine (60.0%) (Table I).

Correlation analyses

Serum Wnt3A levels at diagnosis positively correlated with cross-sectional BVAS (r=0.243, p=0.030), FFS (r=0.245, p=0.028), ESR (r=0.488, $p<0.001$), and CRP levels (r=0.311, p=0.006). Conversely, the levels were inversely correlated with the cross-sectional SF-36 PCS (r = -0.248, p=0.026), and serum albumin levels (r= -0.441, $p<0.001$), respectively (Fig. 1).

Table I. Characteristics of patients with AAV at diagnosis and during follow-up (n=80).

Variables	Values
At the time of diagnosis	
Demographic data	
Age (years)	63.5 (52.0~73.8)
Male sex (n, (%))	32 (40.0)
Female sex (n, (%))	48 (60.0)
AAV subtypes (n, (%))	
MPA	39 (48.8)
GPA	24 (30.0)
EGPA	17 (21.3)
ANCA positivity (n, (%))	
MPO-ANCA (or P-ANCA) positive	45 (56.3)
PR3-ANCA (or C-ANCA) positive	12 (15.0)
Both ANCA positive	3 (3.8)
ANCA negative	26 (32.5)
Systemic items of BVAS	
General manifestation	0 (0~0)
Cutaneous manifestation	0 (0~0)
Mucous and ocular manifestation	0 (0~0)
Otorhinolaryngologic manifestation	1.0 (0~1.0)
Pulmonary manifestation	2.0 (0~4.0)
Cardiovascular manifestation	0 (0~0)
Gastrointestinal manifestation	0 (0~0)
Renal manifestation	0 (0~7.5)
Nervous systemic manifestation	0 (0~3.5)
AAV-specific indices	
BVAS	5.0 (3.0~17.0)
FFS	0 (0~1.0)
SF-36 PCS	52.2 (34.5~67.7)
SF-36 MCS	55.3 (39.7~72.6)
VDI	3.0 (2.0~4.0)
Comorbidities (n, (%))	
Type 2 diabetes mellitus	17 (21.3)
Hypertension	26 (32.5)
Dyslipidaemia	14 (17.5)
Acute-phase reactants	
ESR (mm/hr)	19.0 (7.0~72.5)
CRP (mg/L)	3.6 (0.8~27.0)
Laboratory results	
White blood cell count (/mm^3)	7,710.0 (5,965.0~10,545.0)
Haemoglobin (g/dL)	12.2 (10.2~13.6)
Platelet count (x1,000/mm^3)	246.0 (193.0~360.0)
Fasting glucose (mg/dL)	94.0 (88.0~109.0)
Blood urea nitrogen (mg/dL)	19.3 (13.8~28.7)
Serum creatinine (mg/dL)	0.8 (0.6~1.6)
eGFR CKD-EPI (mL/min/1.732m^2)	92.2 (36.4~106.3)
Total serum protein (g/dL)	6.8 (6.4~7.3)
Serum albumin (g/dL)	4.2 (3.6~4.4)
Alkaline phosphatase (IU/mL)	70.0 (58.0~92.0)
Aspartate aminotransferase (IU/mL)	20.0 (16.0~25.8)
Alanine aminotransferase (IU/mL)	17.0 (11.0~26.3)
Total bilirubin (mg/dL)	0.6 (0.4~0.9)
C3 (mg/dL)	113.5 (97.5~126.3)
C4 (mg/dL)	25.4 (20.2~31.0)
Serum Wnt3A (pg/mL)	322.7 (231.5~442.6)
During follow-up	
Poor outcome (n, (%)	
All-cause mortality	6 (7.5)
Relapse	12 (15.0)
ESKD	19 (23.8)
CVA	4 (5.0)
ACS	1 (1.3)
Follow-up duration based on each poor outcome (months)	
All-cause mortality	26.6 (11.9~44.7)
Relapse	22.0 (6.4~32.5)
ESKD	26.2 (8.7~44.7)
CVA	26.4 (11.0~42.0)
ACS	26.4 (11.8~42.0)
Medications	
Glucocorticoids	79 (98.8)
Cyclophosphamide	53 (66.3)
Rituximab	16 (20.0)
Mycophenolate mofetil	20 (25.0)
Azathioprine	48 (60.0)
Tacrolimus	7 (8.8)
Methotrexate	3 (3.8)

Values are expressed as a median (25~75 percentile) or n (%).
ANCA: antineutrophil cytoplasmic antibody; AAV: ANCA-associated vasculitis; MPA: microscopic polyangiitis; GPA: granulomatosis with polyangiitis; MPO: myeloperoxidase; P: perinuclear; PR3: proteinase 3; C: cytoplasmic; BVAS: the Birmingham vasculitis activity score; FFS: the five-factor score; SF36: 36-item short form survey; PCS: physical component summary; MCS: mental component summary; VDI: vasculitis damage index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; eGFR CKD-EPI: estimated glomerular filtration rate by the chronic kidney disease epidemiology collaboration; C3: complement 3; C4: complement 4; ESKD: end-stage kidney disease; CVA: cerebrovascular accident; ACS: acute coronary syndrome.

Cut-off of Wnt3A and RR for high BVAS

In the ROC curve analysis, the AUC of serum Wnt3A levels at diagnosis for the cross-sectional high BVAS was 0.651, and the confidence interval (CI) was 0.505 to 0.796. Establishing a serum Wnt3A level of 411.7 pg/mL at diagnosis as the threshold for identifying high BVAS yielded sensitivity and specificity of 55.6%, and 79.2%, respectively (Fig. 2A). When patients were divided into two groups according to serum Wnt3A ≥411.7 pg/mL, high BVAS was identified more frequently in patients with serum Wnt3A ≥411.7 pg/mL than in those with serum Wnt3A <411.7 pg/mL (57.7% *vs*. 22.2%, p=0.002). Additionally, patients with serum Wnt3A ≥411.7 pg/mL exhibited a significantly higher risk for high BVAS than those with lower levels (RR 4.773, 95% CI 1.741, 13.083) (Fig. 2B).

Cut-off of Wnt3A and RR for the progression to ESKD

ROC curve analysis revealed that serum Wnt3A levels at diagnosis tended to be associated with only ESKD among the five poor outcomes during follow-up (p=0.055). Therefore, we investigated the clinical implications of serum Wnt3A levels at diagnosis to predict advancement to ESKD during follow-up in patients with AAV. In the ROC curve analysis, the AUC of serum Wnt3A levels at ESKD diagnosis during follow-up was 0.646, and the C) was 0.498–0.794. When the cut-off value for the serum levels of Wnt3A at diagnosis for the progression to ESKD during follow-up was set as 407.1 pg/mL, the sensitivity and specificity were 57.9%, and 73.8%, respectively (Fig. 3A). When patients were divided into two groups according to serum Wnt3A ≥407.1 pg/mL, the advancement to ESKD was observed more often in patients with serum Wnt3A ≥407.1 pg/mL than those with lower levels (40.7% *vs*. 15.1%, p=0.011). Additionally, patients with serum Wnt3A ≥407.1 pg/mL exhibited a significantly higher risk for the progression to ESKD than those with lower serum Wnt3A levels (RR 3.867, 95% CI 1.320, 11.327) (Fig. 3B).

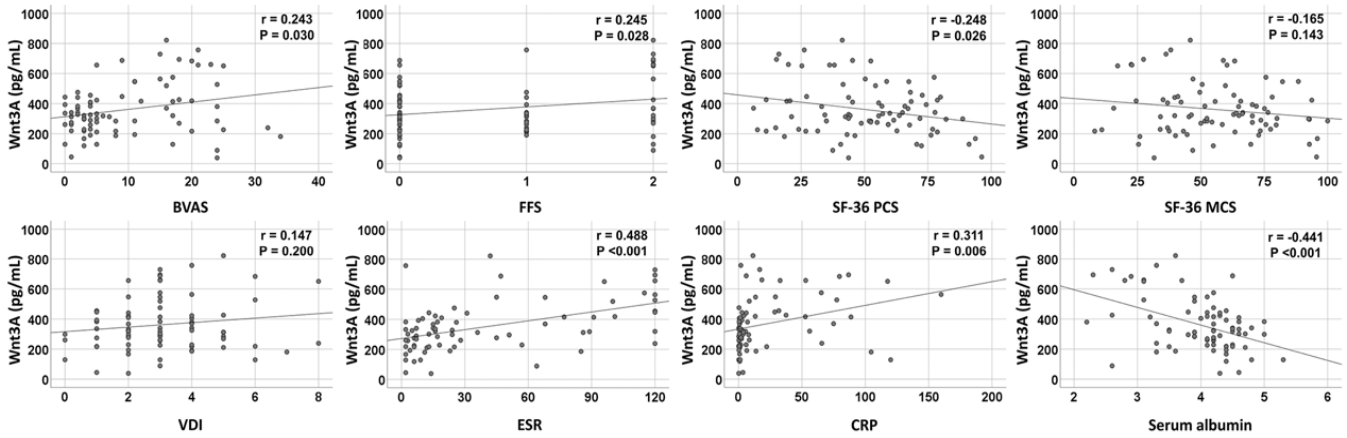

Fig. 1. Correlation analyses.
At diagnosis, serum Wnt3A levels significantly correlated with BVAS, FFS, SF-36 PCS, ESR, CRP, and serum albumin levels in patients with AAV.
BVAS: Birmingham Vasculitis Activity Score; FFS: five-factor score; SF-36: 36-item short-form survey; PCS: physical component summary; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; ANCA: antineutrophil cytoplasmic antibody; AAV: ANCA-associated vasculitis.

Cumulative ESKD-free survival rates

When using the cut-off of serum levels of Wnt3A at diagnosis for the advancement to ESKD, patients with serum Wnt3A ≥407.1 pg/mL exhibited a significantly lower cumulative ESKD-free survival rate than those with serum Wnt3A <407.1 pg/mL (p=0.004). Conversely, when using the cut-off of serum levels of Wnt3A at diagnosis for identifying the cross-sectional high BVAS, patients with serum Wnt3A ≥411.7 pg/mL also exhibited a significantly lower cumulative ESKD-free survival rate than those with serum Wnt3A <411.7 pg/mL (p=0.014) (Fig. 4).

Cox proportional analyses

In the univariable Cox analysis with variables at diagnosis for the progression to ESKD, female sex, BVAS, ESR, CRP, haemoglobin, serum creatinine, serum albumin, and serum levels of Wnt3A especially those ≥407.1 pg/mL were significantly associated with ESKD, respectively. However, eGFR at diagnosis was not significantly associated with future progression to ESKD in patients with AAV in this study. In the multivariate Cox analysis, ESR and serum creatinine levels were significantly associated with ESKD. However, neither serum levels of Wnt3A nor serum Wnt3A ≥407.1 pg/mL were associated with ESKD (Table II).

Discussion

Given the role of Wnt3A as a new

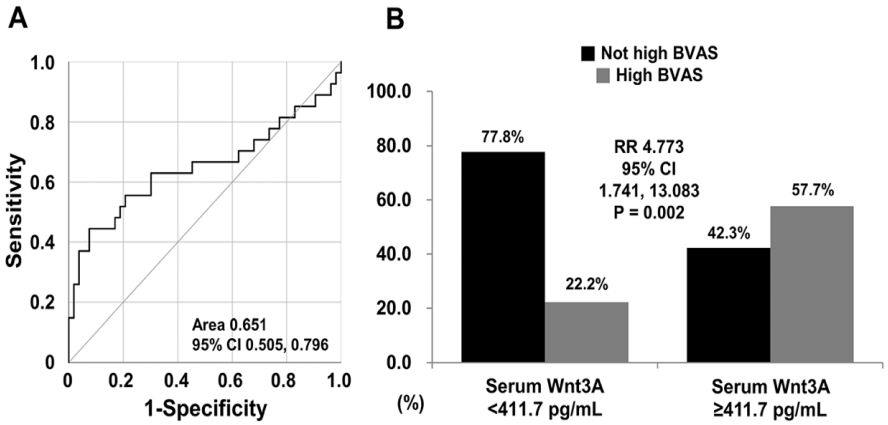

Fig. 2. Cut-off of Wnt3A and relative risk for high BVA.
When the cut-off of serum levels of Wnt3A at diagnosis for the cross-sectional high BVAS was determined as 411.7 pg/mL, the sensitivity and specificity were 55.6%, and 79.2%, respectively. Patients with serum Wnt3A ≥411.7 pg/mL frequently exhibited high BVAS and displayed a significantly higher risk for high BVAS than the risk observed in those with lower levels.
BVAS: the Birmingham vasculitis activity score; RR: relative risk; CI: confidence interval.

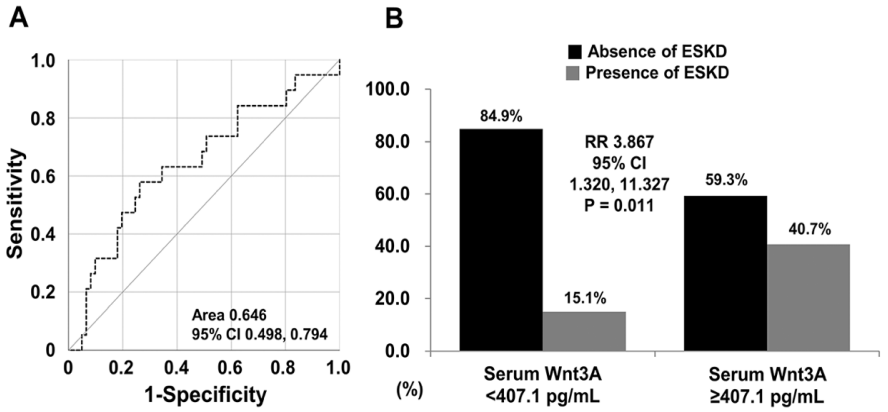

Fig. 3. Cut-off of Wnt3A and relative risk for the advancement to ESKD.
When establishing the cut-off of serum levels of Wnt3A at diagnosis predicting the progression to ESKD during follow-up as 407.1 pg/mL, the sensitivity and specificity were 57.9%, and 73.8%, respectively. Patients with serum Wnt3A ≥407.1 pg/mL reported a high incidence of ESKD and exhibited a significantly elevated risk for the advancement to ESKD than those with lower levels.
ESKD: end-stage kidney disease; RR: relative risk; CI: confidence interval.

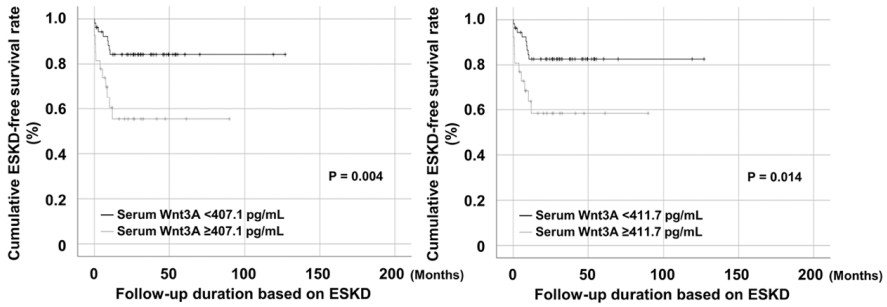

Fig. 4. Comparison of cumulative ESKD-free survival rates.
Patients with serum Wnt3A ≥407.1 pg/mL as well as those with serum Wnt3A ≥411.7 pg/mL exhibited significantly lower cumulative ESKD-free survival rates than those with lower levels.
ESKD: end-stage kidney disease.

biomarker in cancers and the connection of Wnt3A with pivotal inflammatory cytokines, this study investigated the clinical potential of serum Wnt3A levels in estimating cross-sectional activity and predicting poor outcomes in patients with AAV. Moreover, the study obtained several interesting findings. First, serum levels of Wnt3A at diagnosis were correlated with the cross-sectional BVAS and serum Wnt3A ≥411.7 pg/mL exhibited an increased risk for high BVAS. These results suggest that serum Wnt3A levels may be useful for estimating the cross-sectional activity in patients with AAV. Second, in addition to BVAS, serum levels of Wnt3A at diagnosis were significantly correlated with cross-sectional acute-phase reactants, ESR, CRP, and serum albumin. These results suggest that serum Wnt3A levels are not specific to BVAS and may reflect the general burden of inflammation. Third, the serum levels of Wnt3A at diagnosis were associated with AAV exacerbation, leading to ESKD. Particularly, serum Wnt3A ≥407.1 pg/mL also demonstrated an elevated risk for progression to ESKD. Additionally, patients with serum Wnt3A ≥407.1 pg/mL exhibited a significantly lower cumulative ESKD-free survival rate than those with serum Wnt3A <407.1 pg/mL. These results suggest that serum levels of Wnt3A at diagnosis could be useful in predicting progression to ESKD during follow-up. However, the predictive capability of Wnt3A levels for ESKD occurrence does not match that of the initial levels of serum creatinine. Taken together

Table II. Cox hazards model analyses of variables at diagnosis for ESKD during follow-up in patients with AAV.

Variables at diagnosis	Univariable			Multivariable (Serum levels of Wnt3A)			Multivariable (Serum Wnt3A ≥407.1 pg/mL)		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age (years)	1.013	0.981, 1.046	0.431						
Male sex	0.261	0.076, 0.895	0.033						
Female sex	3.835	1.117, 13.164	0.033	1.017	0.795, 20.294		3.711	0.732, 18.815	0.113
MPO-ANCA (or P-ANCA) positivity	0.957	0.742, 5.160	0.175						
PR3-ANCA (or C-ANCA) positivity	0.636	0.147, 2.753	0.545						
BVAS	1.046	0.998, 1.095	0.058	1.076	0.969, 1.193	0.169	1.070	0.964, 1.188	0.202
FFS	1.302	0.761, 2.230	0.336						
VDI	0.950	0.716, 1.261	0.724						
Type 2 diabetes mellitus	0.455	0.105, 1.970	0.292						
Hypertension	2.052	0.834, 5.053	0.118						
Dyslipidaemia	1.425	0.473, 4.295	0.529						
ESR	1.016	1.005, 1.028	0.004	1.037	1.013, 1.063	0.003	1.029	1.005, 1.053	0.019
CRP	1.016	1.005, 1.026	0.002	0.983	0.954, 1.014	0.277	0.981	0.953, 1.011	0.213
White blood cell count (/mm^3)	1.025	0.938, 1.120	0.585						
Haemoglobin (g/dL)	0.770	0.619, 0.958	0.019	1.335	0.801, 2.224	0.268	1.231	0.741, 2.043	0.422
Platelet count (x1,000/mm^3)	1.001	0.99, 1.004	0.291						
Fasting glucose (mg/dL)	1.002	0.991, 1.013	0.754						
Blood urea nitrogen (mg/dL)	1.014	0.988, 1.039	0.292						
Serum creatinine (mg/dL)	1.323	1.074, 1.629	0.008	2.758	1.563, 4.867	<0.001	2.455	1.424, 4.234	0.001
Total serum protein (g/dL)	1.677	0.875, 3.215	0.119						
Serum albumin (g/dL)	0.548	0.284, 1.059	0.074	2.071	0.373, 11.483	0.405	2.144	0.417, 11.034	0.362
eGFR CKD-EPI (mL/min/1.732m^2)	0.991	0.979, 1.003	0.130						
Aspartate aminotransferase (IU/mL)	1.007	0.966, 1.049	0.753						
Alanine aminotransferase (IU/mL)	0.999	0.977, 1.021	0.901						
C3 (mg/dL)	1.012	0.994, 1.031	0.194						
C4 (mg/dL)	0.995	0.948, 1.044	0.834						
Serum levels of Wnt3A (pg/mL)	1.003	1.000, 1.005	0.025	2.071	0.373, 11.483	0.149			
Serum Wnt3A ≥ 407.1 pg/mL	3.502	1.403, 8.744	0.007				1.193	0.261, 5.454	0.820

ESKD: end-stage kidney disease; ANCA: antineutrophil cytoplasmic antibody; AAV: ANCA-associated vasculitis; MPO: myeloperoxidase; P: perinuclear; PR3: proteinase 3; C: cytoplasmic; BVAS: the Birmingham vasculitis activity score; FFS: the five-factor score; SF36: 36-item short form survey; PCS: physical component summary; MCS: mental component summary; VDI: vasculitis damage index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; eGFR CKD-EPI: estimated glomerular filtration rate by the chronic kidney disease epidemiology collaboration; C3: complement 3; C4: complement 4.

with these results, we conclude that serum levels of Wnt3A may have clinical significance as a novel biomarker for not only estimating cross-sectional activity but also predicting advancement to ESKD during follow-up in patients with AAV.

Given that this was a retrospective study using stored serum, we acknowledge the limitation of not being able to prove the mechanism by which serum levels of Wnt3A can estimate the cross-sectional activity expressed by BVAS in patients with AAV through alterations in the intracellular Wnt signalling pathway (13, 14). Instead, we aimed to reveal organ-specific correlations by determining which serum levels of Wnt3A were highly correlated among the nine systemic items comprising the BVAS (7). Serum Wnt3A levels at diagnosis significantly correlated with the cross-sectional total scores for general (r=0.226, p=0.044) and renal manifestations (r=0.305, p=0.006). Of the four general manifestation sub-items, none significantly correlated with serum Wnt3A levels at diagnosis. Conversely, of the seven sub-items of the renal manifestation item, both the sub-items of proteinuria >1+ (r=0.292, p=0.009) and serum creatinine levels 2.83 to 5.64 mg/dL (r=0.253, p=0.023) were significantly correlated with serum levels of Wnt3A at diagnosis (33). These results demonstrate that the kidney-related sub-items of the BVAS play a significant role in estimating the cross-sectional activity represented by the BVAS in patients with AAV. Therefore, we realised that it was necessary to shift our focus to a more specific topic, the link between the Wnt3A signalling pathway and nephropathy, from the broad scope of the link between the Wnt3A signalling pathway and AAV (34).

Several previous studies have demonstrated the clinical role of the Wnt3A signalling pathway in the pathogenesis of nephropathies:

i. Wnt signalling pathway activity is enhanced in glomerulonephritis and further involved in the morphological alterations in the kidneys in lupus nephritis. Therefore, the Wnt signalling pathway was suggested as a critical contributor to the development of lupus nephritis (35).

ii. The participation of Wnt signalling pathway in renal fibrosis pathogenesis has been demonstrated (36).

iii. Furthermore, the Wnt3A signalling pathway contributed to kidney fibrosis in type II cardiorenal syndrome by inducing multiple components of the renin-angiotensin system in both cardiomyocytes and cardiac fibroblasts (37).

Based on the association between serum Wnt3A and proteinuria, increased serum creatinine, and the association between the Wnt signalling pathway and the pathogenesis of nephropathies mentioned above, we hypothesised that elevated serum levels of Wnt3A at diagnosis may predict the advancement to future ESKD. This prediction may not only reflect the current extent of kidney inflammation and dysfunction but also micro-level kidney damage that has the potential to progress to chronic renal failure over time. However, the predictive potential of serum Wnt3A levels for advanced ESKD was not as high as that of serum creatinine levels at diagnosis (Table II).

As MPA among the three subtypes of AAV is more susceptible to ANCA-associated GN, we included only patients with MPA and investigated the association of serum levels of Wnt3A at diagnosis with advancement to ESKD during follow-up. In ROC curve analysis, the AUC of serum Wnt3A levels at diagnosis for ESKD occurrence during follow-up was statistically significant (AUC 0.742, 95% CI 0.548, 0.936). The optical cut-off of serum Wnt3A levels at diagnosis for progression to ESKD was obtained as 554.8 pg/mL (sensitivity, 62.5; specificity, 81.7%). Patients with serum Wnt3A ≥554.8 pg/mL exhibited a high proportion of ESKD and a higher risk for ESKD compared to the risk observed in those with lower levels (62.5% *vs.* 12.9%, p=0.009; RR 11.250, 95% CI 1.906, 66.393). Patients with serum Wnt3A ≥554.8 pg/mL also exhibited a significantly lower cumulative ESKD-free survival rate compared to the rate in those with lower levels (p=0.002) (Supplementary Fig. S1). Additionally, in the univariable Cox analysis with variables at diagnosis for the advancement to ESKD, both serum levels of Wnt3A and serum Wnt3A ≥554.8 pg/mL were significantly associated with ESKD, along with BVAS, FFS, ESR, CRP, haemoglobin, platelet count, serum creatinine, and alanine aminotransferase. However, in multivariate Cox analysis, none, including serum creatinine, was significantly and independently associated with ESKD (Supplementary Table S1). Therefore, we concluded that serum Wnt3A levels might be associated with progression to ESKD, but could not surpass the predictive ability of serum creatinine, regardless of AAV subtype.

What advantages does quantifying serum Wnt3A levels at diagnosis offer compared to assessing BVAS for cross-sectional activity or measuring serum creatinine levels for advancement to ESKD during follow-up in patients with AAV? Moreover, the results of this study can be applied to patients newly diagnosed with AAV in clinical settings. First, in terms of the current status of inflammation, serum Wnt3A levels at diagnosis can be considered a biomarker reflecting the cross-sectional burden of inflammation. This is because serum levels of Wnt3A not only correlate with BVAS but also with traditional inflammatory markers, such as ESR, CRP, and serum albumin (38). Therefore, quantifying the serum levels of Wnt3A at diagnosis in patients who are newly diagnosed with AAV and have never been exposed to immune-modulating drugs could be useful for estimating not only the current AAV activity but also the cross-sectional systemic burden of inflammation. Second, in terms of ESKD occurrence, although the potential of serum Wnt3A levels as a predictor of progression to ESKD varied, they were not as predictive as serum creatinine at diagnosis. On the other hand, interestingly, serum levels of Wnt3A were not significantly correlated with serum creatinine at diagnosis (r=0.096). As elevated serum creatinine levels reflect impaired kidney function, accepting that they can predict the transition to CRF or ESKD is easy. In contrast, the fact that Wnt3A can predict the transition to ESKD

without maintaining a proportional relationship with serum creatinine suggests that a hidden mechanism exists that reflects ESKD-driving factors other than serum creatinine. In terms of type 2 diabetes mellitus, the Wnt3a pathway is known to be linked to the development of diabetic nephropathy (39) and thus, it may be clinically meaningful to unveil the association between the presence of type 2 diabetes mellitus and serum Wnt3a levels at diagnosis. Because the presence of type 2 diabetes mellitus is one of the categorical variables, we first compared serum Wnt3A levels at diagnosis between patients with and without type 2 diabetes mellitus using a Mann-Whitney U-test and found no significant difference between the two groups (341.0 *vs*. 319.4 pg/mL, p=0.702). Additionally, we conducted a Pearson's correlation analysis and observed no significant correlation between the two variables (r = 0.048, p=0.670) either.

It has been known that the presence of type 2 diabetes mellitus is a significant risk factor for chronic kidney diseases (diabetic nephropathy) and thus, it was expected that the initial type 2 diabetes mellitus might have an influence on the progression to ESKD during the disease course of type 2 diabetes mellitus in patients with AAV like the general population. However, even though 21.3% of patients had diabetes at the time of AAV diagnosis, in Table II, type 2 diabetes mellitus was not significantly associated with future progression to ESKD in this study. We inferred that the inaccurate information on the duration of type 2 diabetes mellitus might be the cause of this discrepancy. This was because diabetes nephropathy and its associated progression to ESKD are closely related to the duration of type 2 diabetes mellitus. In other words, the longer the duration of type 2 diabetes mellitus, the greater the probability of the transition from subclinical renal damage to clinical renal damage. Nevertheless, this study could not accurately collect the duration of type 2 diabetes mellitus due to the limitation of a retrospective study design. Additionally, we inferred that the relatively short follow-up period of this study might be another cause of this discrepancy.

On the other hand, to indirectly assess the longitudinal effect of type 2 diabetes mellitus on the decline of renal function, we compared the median cross-sectional serum creatinine and eGFR CKD-EPI at AAV diagnosis between patients with type 2 diabetes mellitus and those without. We found no significant differences in serum creatinine and eGFR CKD-EPI between the two groups (1.0 *vs*. 0.8 mg/dL, p=0.186, and 75.8 *vs*. 92.8 mL/min/1.732m^2, p=0.178, respectively). The strength of the present study is that it is the first to demonstrate the clinical potential of serum Wnt3A levels at diagnosis for estimating the cross-sectional activity and partially predicting the advancement to ESKD during follow-up in patients with AAV as well as those with MPA.

This study had several limitations. The number of patients with AAV was not sufficiently large to generalise the results of this study and apply them immediately to patients newly diagnosed with AAV in real clinical practice. The retrospective design did not provide sufficient laboratory data for diverse analyses. In particular, it would have been better to discover the association between the initial serum Wnt3A levels and renal histology at diagnosis; however, it could not be done due to the relatively small number of patients in whom subgroup analysis based on the histological classes could be performed. In addition, as the study only utilised sera obtained at diagnosis, the dynamic results of the serum levels of Wnt3A were not available. A future prospective study with an increased number of patients and dynamic data, including serum levels of Wnt3A and BVAS, is expected to provide reliable and clinically significant information on the serum levels of Wnt3A in patients with AAV.

In conclusion, this study is the first to reveal that serum Wnt3A levels at diagnosis could estimate the cross-sectional activity of AAV. Furthermore, the study also demonstrated the potential of serum Wnt3A levels for predicting advancement to ESKD during follow-up in patients with AAV.

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