Serum galectin-9 could be a potential biomarker in assessing the disease activity of antineutrophil cytoplasmic antibody-associated vasculitis

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Abstract

Objective

Serum galectin levels have been reported to be associated with the activity in autoimmune diseases. This study investigated whether serum levels of galectin (Gal)-1, Gal-3, and Gal-9 could be used as biomarkers in assessing the disease activity of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV).

Methods

Eighty AAV patients were selected for inclusion in our AAV cohort. AAV-specific indices and clinical and laboratory data were assessed on the same day when blood samples were obtained from the patient and serum levels of Gal-1, Gal-3, and Gal-9 were measured by ELISA from obtained sera. High disease activity was defined as Birmingham vasculitis activity score (BVAS) ≥ 12. The optimal cut-off value of galectins was extrapolated by receiver operator characteristic analysis and linear and logistic regression analyses were performed to evaluate the association between Gal-3, Gal-9, and BVAS.

Results

The median values of BVAS, Gal-1, Gal-3, and Gal-9 were 8.0, 38.1 ng/mL, 12.4 ng/mL, and 1017.7 ng/mL, respectively. Serum Gal-3 and Gal-9 levels were correlated with BVAS (r=0.375 and r=0.462), while only serum Gal-9 levels were independently associated with BVAS (β=0.250) in linear regression analyses. Serum Gal-9 ≥10.28 ng/mL was also associated with high activity of AAV (odds ratio 5.303) in multivariable logistic regression analysis. In addition, serum Gal-1, Gal-3, and Gal-9 levels were found to differ according to ANCA positivity status and the presence of renal manifestations.

Conclusion

These results suggest the potential possibility of serum Gal-9 levels in assessing AAV disease activity.

Key words

galectin-9, antineutrophil cytoplasmic antibody-associated vasculitis, activity, Birmingham vasculitis activity score, biomarker

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Introduction

Galectins belong to the family of animal lectins that bind to beta-galactoside; these lectins possess an evolutionarily conserved carbohydrate recognising domain (1). Galectins are synthesized in the free ribosomes of the cytoplasm and the expression in various tissues has been shown to be modulated under pathological conditions such as cancers and chronic inflammatory disorders (2). Previous studies have demonstrated that galectins are critical regulators in the maintenance of homeostasis via modulating cellular apoptosis and proliferation, cell cycle, and immune response (3). Interestingly, they are known to play a pleiotropic role in immunity through influencing the cells involved in both adaptive and innate immune systems, such as lymphocytes, macrophages, natural killer cells, and dendritic cells. To date, twelve different galectins have been identified in humans; these are distinguished on the basis of their structure and binding affinity to beta-galactose-containing glycans (4). Given that the galectin-glycoprotein interaction plays an important role in mediating cellular signalling pathways, galectins have gained increasing interest in human diseases. Among the discovered galectins, galectin (Gal)-1, -3 (Gal-3), and -9 (Gal-9) have been extensively studied per the existing literature and have been demonstrated to affect differentiation of T- and B- lymphocytes via influencing the process of apoptosis, migration, activation, and maturation (3, 5).

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), together with immune complex vasculitis, is known to comprise a group of small-vessel vasculitis. AAV is characterised by necrotising vasculitis with few or no immune deposits and is categorised into three distinct subtypes, including microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), and eosinophilic granulomatosis with polyangiitis (EGPA) (6, 7). In inflammatory conditions, Gal-1, Gal-3, and Gal-9 have been shown to regulate and modulate the functions of T_H1, T_H17, Treg, T_{FH}, and B cells which also play important roles in the pathogenesis of AAV (3, 4). Therefore, it can be theoretically inferred that Gal-1, Gal-3, and Gal-9 are associated with the disease activity of AAV. By far, there have been several studies regarding the clinical significance of serum Gal-1, Gal-3, and Gal-9 levels in assessing the disease activity in autoimmune diseases. Serum Gal-1 and Gal-3 levels were found to be significantly higher in patients with inflammatory bowel diseases than in healthy controls (8). Serum Gal-1 level was significantly correlated with the disease activity of rheumatoid arthritis (9). Additionally, serum Gal-9 level was significantly correlated with the disease activity of systemic lupus erythematosus (SLE) (10). Furthermore, high levels of serum Gal-9 were significantly associated with renal involvement and the increase of Gal-3-binding protein levels predicted occurrence of venous thromboembolism in SLE (10, 11). However, no study has investigated the association between serum levels of galectins and the disease activity of AAV to date. Therefore, we investigated whether serum levels of Gal-1, Gal-3, and Gal-9 could be used as effective biomarkers in assessing the disease activity of AAV.

Materials and methods

Patients with AAV

In this study, 80 patients with AAV, those who were enrolled in the Severance Hospital ANCA-associated VasculitidEs (SHAVE) cohort with available sera and patient data, were included. This cohort is a prospective and observational cohort of patients with MPA, GPA, and EGPA, formed in November 2016. AAV was diagnosed and confirmed in all patients at Division of Rheumatology, Department of Internal Medicine, Yonsei University College of Medicine and Severance Hospital. Incident and prevalent AAV cases were both recruited and all patients fulfilled the criteria of the 2007 European Medicines Agency algorithms for AAV and polyarteritis nodosa (the 2007 EMA algorithm) and the 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides (the 2012 CHCC definitions) (6, 7). Basically, patients included in the SHAVE cohort are regularly (every three to six

months) assessed for AAV-specific indices and had their blood collected. The selected patients did not have serious infectious diseases, malignancies, or autoimmune diseases other than AAV that could affect levels of galectins. This study was approved by the Institutional Review Board of Severance Hospital (4-2016-0901) and patients who agreed to participate in the cohort provided written informed consent at the time of first blood sampling.

Clinical and laboratory data

At blood sampling, age, sex, and follow-up duration were collected as demographic data. AAV subtypes were classified into MPA, GPA, and EGPA; clinical manifestations were reviewed based on the categories consisting the Birmingham vasculitis activity score (BVAS) (12). Myeloperoxidase (MPO)-ANCA and proteinase 3 (PR3)-ANCA were measured using the novel anchor-coated highly sensitive Phadia EliA kit using the Phadia250 analyser. We used immunoassays as the primary screening method for ANCA; however, when patients were found to be negative for ANCA by an antigen-specific assay but positive for perinuclear (P)-ANCA or cytoplasmic (C)-ANCA with an indirect immunofluorescence assay, they were also considered to have MPO-ANCA or PR3-ANCA (13). Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were assessed as acute-phase reactants. The immunosuppressive drugs, currently prescribed to the patients, were identified using the Korean Drug Utilisation Review system.

AAV-specific indices and sera collection

AAV-specific indices, assessed in the SHAVE cohort, were used: BVAS (v. 3) and five-factor score (FFS) were calculated to assess the cross-sectional activity and prognosis (12, 14). Vasculitis damage index (VDI) was evaluated to estimate the extent of organ damage (15). The Korean version of the Short-Form 36-Item Health Survey Physical and Mental Component Summaries (SF-36 PCS and SF-36 MCS) were collected to evaluate the functional status (16). BVAS, FFS, and VDI forms were primarily completed by the attending physician, whereas SF-36 PCS and SF-36 MCS were filled by the patients visiting the clinic. On the day of hospital visit, whole blood sample was obtained from each patient with a consent and sera was isolated and stored at -80 °C until use.

Measurement of serum galectins and cytokines and definition of high activity

Serum Gal-1, Gal-3, Gal-9, interleukin (IL)-6, and IL-8 levels were quantified from stored sera using Human Magnetic Luminex® assay (R&D Systems, USA) following the manufacturer's instruction. We categorised AAV patients into three groups based on the tertile of BVAS and defined the lower limit of the highest tertile as the cut-off for high activity of AAV (BVAS ≥12). Serum obtained from 40 patients with SLE and healthy controls (HC) were also analysed to compare serum galectin levels with AAV patients.

Statistical analyses

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software (v. 25 for Windows; IBM Corp., Armonk, NY, USA). Continuous variables are expressed as medians with interquartile ranges, whereas categorical variables are expressed as numbers (percentages). Significant differences between the two and three continuous variables were compared using Mann-Whitney and Kruskal-Wallis tests. The correlation coefficient (r) between the two variables was obtained using the Pearson correlation analysis. The standardised correlation coefficient (β) was obtained through the multivariable linear regression analysis using variables with statistical significance in the univariable analysis. The odds ratio (OR) was obtained using the multivariable logistic regression analysis. The optimal cut-off value of galectins was extrapolated by performing receiver operator characteristic curve analysis. Assessment of relative risk (RR) for the cut-off was analysed using contingency tables and the chi-square test.

Results

Patients' characteristics

The median age of the included patients was 63 years, 42.5% of them were male, and the median follow-up duration was 1.0 months. Among them, 40, 25, and 15 patients had MPA, GPA, and EGPA, respectively. ANCA was detected in 57 patients. The median values of BVAS, FFS, VDI, SF-36 PCS, and SF-MCS were 8.0, 1.0, 3.0, 53.8, and 55.6, respectively. The most common observed clinical manifestation was pulmonary (58.8%), followed by renal (53.8%) and otorhinolaryngologic (42.5%). The median serum levels of Gal-1, Gal-3, Gal-9, IL-6, and IL-8 were 38.1 ng/mL, 12.6 ng/mL, 9.8 ng/mL, 3.4 pg/mL, and 21.4 pg/mL. Glucocorticoid and azathioprine were administered to 67 (83.8%) and 30 (37.5%) patients (Table I). Comparison of serum galectins with SLE patients and HC revealed that serum Gal-1 and Gal-3 levels were significantly higher in patients with AAV compared to those with SLE and HC (all $p<0.001$). On the other hand, serum Gal-9 levels in patients with AAV and SLE was increased compared to HC ($p<0.001$) (Supplementary Fig. S1).

Correlation between BVAS and serum levels of galectins

BVAS was found to be significantly correlated with serum levels of Gal-3 (r=0.375) and Gal-9 (r=0.462). FFS was significantly correlated with serum levels of Gal-1 (r=0.448), Gal-3 (r=0.322), and Gal-9 (r=0.405). However, VDI was not correlated with any of these parameters. Both SF-36 PCS and MCS were inversely correlated with serum levels of Gal-3 (r=-0.272, r=-0.271) and Gal-9 (r=-0.257, r=-0.252) (Table II). This result indicates that serum levels of galectins might reflect the disease activity, prognostic, and functional indices. Among galectins and cytokines, serum Gal-1 level was significantly correlated with serum Gal-3 (r=0.284), Gal-9 (r=0.571), and IL-8 (r=0.278) levels; the correlation coefficient between serum Gal-3 and Gal-9 levels was the highest (r=0.607, $p<0.001$) (Supplementary Table S1).

Linear regression analyses of variables with BVAS

In the univariable analysis, ESR, CRP, serum Gal-3, and Gal-9 levels were significantly associated with BVAS.

In the multivariable analysis with ESR, CRP, serum Gal-3, and Gal-9 levels, only serum Gal-9 level was independently associated with BVAS (β=0.250, *p*=0.047). Serum Gal-3 level also tended to be correlated with BVAS but it did not exhibit statistical significance (β=0.233, *p*=0.068). There was no multicollinearity between serum Gal-3 and Gal-9 levels based on BVAS (Table III).

Cut-off of serum Gal-3 and Gal-9 levels for high activity of AAV

When the cut-off of Gal-3 for high activity of AAV (BVAS ≥12) was set at 13.53 ng/mL, sensitivity and specificity were 55.2% and 72.5% (area 0.644, 95% confidence interval [CI] 0.514, 0.775), respectively. High activity was identified more frequently in AAV patients with serum Gal-3 ≥13.53 ng/mL compared to those with Gal-3 <13.53 ng/mL (53.3% *vs*. 26.0%, *p*=0.014, RR 3.253, 95% CI 1.251, 8.461) (Fig. 1A). Moreover, when the cut-off of Gal-9 for high activity of AAV was set at 10.28 ng/mL, sensitivity and specificity were 75.9% and 68.6% (area 0.718, 95% CI 0.600, 0.836), respectively. Patients with serum Gal-9 ≥10.28 ng/mL had greater proportion of those with high activity than in those with Gal-9 <10.28 ng/mL (57.9% *vs*. 16.7%, *p*<0.001, RR 6.875, 95% CI 2.440, 19.373).

Logistic regression analyses based on high activity of AAV

In the univariable analysis, ESR, CRP, Gal-3, and Gal-9 levels were associated with high activity of AAV. In the multivariable analysis, none of them were independently associated with high activity of AAV. Meanwhile, when two cut-offs of serum Gal-3 ≥13.53 ng/mL and Gal-9 ≥10.28 ng/mL were applied to the logistic regression analyses, serum Gal-9 ≥10.28 ng/mL was independently associated with high activity of AAV (OR 5.303, 95% CI 1.643, 17.114), even though serum Gal-3 ≥13.53 ng/mL was not associated with high activity of AAV (Table IV).

Comparison of galectins, IL-6, and IL-8 serum levels based on ANCA positivity and renal involvement

Patients with MPO-ANCA (or P-ANCA) exhibited significantly higher levels of serum Gal-1 (40.0 *vs*. 35.0 ng/mL), Gal-3 (13.3 *vs*. 10.7 ng/mL), and Gal-9 (11.1 *vs*. 8.4 ng/mL) than those without. Meanwhile, patients with PR3-ANCA (or C-ANCA) showed significantly lower levels of serum Gal-3 (8.6 *vs*. 13.1 ng/mL) and Gal-9 (5.6 *vs*. 11.0), but not Gal-1, than those without (Table V). Among the nine clinical manifestations of AAV based on BVAS version 3, serum levels of galectins were found to be distinct only in regard to renal manifestation. Serum levels of Gal-1 (43.8

Table I. Characteristics of 80 patients with AAV.

Variables	Values
Demographic data	
Age (years)	63.0 (51.7-72.0)
Male sex (n, (%))	34 (42.5)
Follow-up duration (months)	1.0 (0.0-22.8)
AAV subtypes (n, (%))	
MPA	40 (50.0)
GPA	25 (31.3)
EGPA	15 (18.8)
ANCA positivity status (n, (%))	
MPO-ANCA (or P-ANCA) positivity	51 (63.7)
PR3-ANCA (or C-ANCA) positivity	9 (11.3)
ANCA negativity	23 (28.7)
AAV-specific indices	
BVAS	8.0 (4.5-15.0)
FFS	1.0 (1.0-2.0)
VDI	3.0 (2.0-4.0)
SF-36 PCS	53.8 (36.3-67.7)
SF-36 MCS	55.6 (43.0-70.8)
Presence of clinical manifestations (n, (%))	
General	29 (36.3)
Cutaneous	8 (10.0)
Mucous/Eye	3 (3.8)
Otorhinolaryngologic	34 (42.5)
Pulmonary	47 (58.8)
Cardiovascular	4 (5.0)
Abdominal	1 (1.3)
Renal	43 (53.8)
Nervous	22 (27.5)
Acute phase reactants	
ESR (mm/hr)	31.5 (11.0-63.5)
CRP (mg/L)	3.0 (0.6-14.0)
Serum levels of galectins and cytokines	
Galectin-1 (ng/mL)	38.1 (27.1-50.7)
Galectin-3 (ng/mL)	12.6 (9.7-14.7)
Galectin-9 (ng/mL)	9.8 (6.5-15.1)
Interleukin-6 (pg/mL)	3.4 (2.3-7.6)
Interleukin-8 (pg/mL)	21.4 (9.2-88.2)
Administered immunosuppressive drugs (n, (%))	
Glucocorticoid	67 (83.8)
Cyclophosphamide	8 (10.0)
Rituximab	1 (1.3)
Azathioprine	30 (37.5)
Mycophenolate mofetil	1 (1.3)
Tacrolimus	2 (2.5)
Methotrexate	3 (3.8)

Values are expressed as median (interquartile range (IQR)) or number (percentage).
AAV: ANCA-associated vasculitis; ANCA: antineutrophil cytoplasmic antibody; MPA: microscopic polyangiitis; GPA: granulomatosis with polyangiitis; EGPA: eosinophilic granulomatosis with polyangiitis; MPO: myeloperoxidase; P: perinuclear; PR3: proteinase 3; C: cytoplasmic; BVAS: Birmingham vasculitis activity score; FFS: five-factor score; VDI: vasculitis damage index; SF-36: short-form 36-item; PCS: physical component summary; MCS: mental component summary; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

vs. 29.7 ng/mL), Gal-3 (13.6 *vs*. 10.6 ng/mL), and Gal-9 (13.7 *vs*. 7.1 ng/mL) in patients with renal manifestation were significantly higher than those in patients without (Table V). Similarly, the total scores of renal manifestations according to BVAS score were significantly correlated with serum levels of Gal-1 (r=0.435, $p<0.001$), Gal-3 (r=0.381, $p<0.001$), and Gal-9 (r=0.503, $p<0.001$) (Supplementary Fig. S2).

Table II. Correlation of serum levels of galectins with AAV-specific indices.

	BVAS	FFS	VDI	SF-36 PCS	SF-36 MCS
Galectin-1	0.002 (0.987)	0.448 (<0.001)	0.129 (0.254)	0.020 (0.857)	0.019 (0.867)
Galectin-3	0.375 (0.001)	0.322 (0.004)	0.128 (0.257)	-0.272 (0.015)	-0.271 (0.015)
Galectin-9	0.462 (<0.001)	0.405 (<0.001)	0.207 (0.066)	-0.257 (0.022)	-0.252 (0.024)

Values are expressed as correlation coefficients (*p*-values).
AAV: ANCA-associated vasculitis; ANCA: antineutrophil cytoplasmic antibody; BVAS: Birmingham vasculitis activity score; FFS: five-factor score; VDI: vasculitis damage index; SF-36: short-form 36-item; PCS: physical component summary; MCS: mental component summary.

Table III. Linear regression analyses of variables with BVAS.

Variables	Univariable			Multivariable		
	Beta	95% CI	*p*-value	Beta	95% CI	*p*-value
Age	0.122	-0.053, 0.182	0.280			
Male sex	-0.142	-5.623, 1.242	0.208			
MPO-ANCA (or P-ANCA) positivity	0.122	-1.606, 5.472	0.280			
PR3-ANCA (or C-ANCA) positivity	-0.016	-5.798, 5.050	0.891			
ESR	0.387	0.038, 0.127	<0.001	0.231	-0.010, 0.109	0.105
CRP	0.359	0.027, 0.105	0.001	0.137	-0.026, 0.077	0.332
Galectin-1	0.002	-0.111, 0.112	0.987			
Galectin-3	0.375	0.306, 1.076	0.001	0.223	0.000, 0.001	0.068
Galectin-9	0.462	0.283, 0.714	<0.001	0.250	0.000, 0.001	0.047
Interleukin-6	0.210	-0.007, 0.304	0.061			
Interleukin-8	-0.132	-0.014, 0.004	0.249			

BVAS: Birmingham vasculitis activity score; MPO: myeloperoxidase; ANCA: antineutrophil cytoplasmic antibody; P: perinuclear; PR3: proteinase 3; C: cytoplasmic; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

Discussion

In this study, we investigated whether serum levels of Gal-1, Gal-3 and Gal-9 could be useful biomarkers in assessing the disease activity of AAV and obtained several novel findings for the first time. First, serum levels of galectins were significantly elevated in patients with AAV compared to HC; furthermore, Gal-3 and Gal-9 levels were significantly correlated with BVAS and correlated with each other. Second, only serum Gal-9 level was independently associated with BVAS (β=0.250) in the linear regression analyses. Third, when the cut-offs of serum levels of Gal-3 and Gal-9 for high activity of AAV were set as 13.53 ng/mL and 10.28 ng/mL, the serum levels of Gal-3 and Gal-9 over the cut-off increased the risks for high activity of AAV to 3.253 and 6.875. Next, serum Gal-9 ≥10.28 ng/mL was independently and significantly associated with high activity of AAV (OR 5.303) in the multivariable logistic regression analysis. Finally, Gal-1, Gal-3, and Gal-9 were found to differ according to MPO-ANCA (or P-ANCA) and PR3-ANCA (or C-ANCA) positivity and the presence of renal manifestations.

AAV is characterised by typical pathogenic ANCAs, produced by B cells and plasma cells owing to the genetic predisposition, environmental stimuli, neutrophil cytoplasmic antigens, and dysfunction in the regulatory function of T cells (17). During acute inflammation, primed neutrophils are activated, translocate across the vessel walls, and exaggerate inflammation through the production of oxygen radicals and degranulation, thereby leading to the recruitment of monocytes and T cells and the formation of granulomas (18). Additionally, the alternative complement pathway is activated, forming an inflammatory amplification loop and eventually aggravating inflammation (19). Although various immune cells are involved in AAV pathogenesis, the roles of T_H1, T_H17, Treg, $T_{FH,}$ and B cells in severe inflammatory conditions are critical. For instance, the imbalance of T_H17/Treg or T_{FH}/T_{FR} cells (20, 21) and dysregulated B cells (22) are known to be critical for the exacerbation of AAV.

Gal-1, Gal-3, and Gal-9 contribute to maintaining the homeostasis of T cell differentiation and activation. In the peripheral immune system, Gal-1 and Gal-3 prevent the spontaneous activation of T cell receptors in naïve and early activated T cells. Gal-1 and Gal-9 induce the apoptosis of effector T cells, T_H1, and T_H17 cells resulting in reduced production of interferon-γ and IL-17; however, they do not affect T_H2 cells and T_H2-specific cytokines. Moreover, Gal-1 and Gal-9 modulate the function of Treg cells and enhance the production of IL-10 (23, 24). Conversely, Gal-3 inhibits B cell differentiation into plasma cells and enhances their immune tolerance (25). These results suggest that Gal-1, Gal-3, and Gal-9 are involved in alleviating inflammation via the regulation of T cells in severe inflammatory conditions. Therefore, it is likely that serum levels of Gal-3 and Gal-9 showed a significant correlation with BVAS in AAV patients as a part of a homeostatic mechanism to reduce vasculitis-associated inflammation. The following hypothesis seems to be partly supported by our observation that serum levels of galectins were higher in patients with AAV compared to HC. However, compared to other autoimmune diseases, the role of serum Gal-1 level in reflecting inflammation was insignificant. Also, serum Gal-9 level was found to be more effective than serum Gal-3 level as a biomarker in assessing the disease activity of AAV in the selected patients.

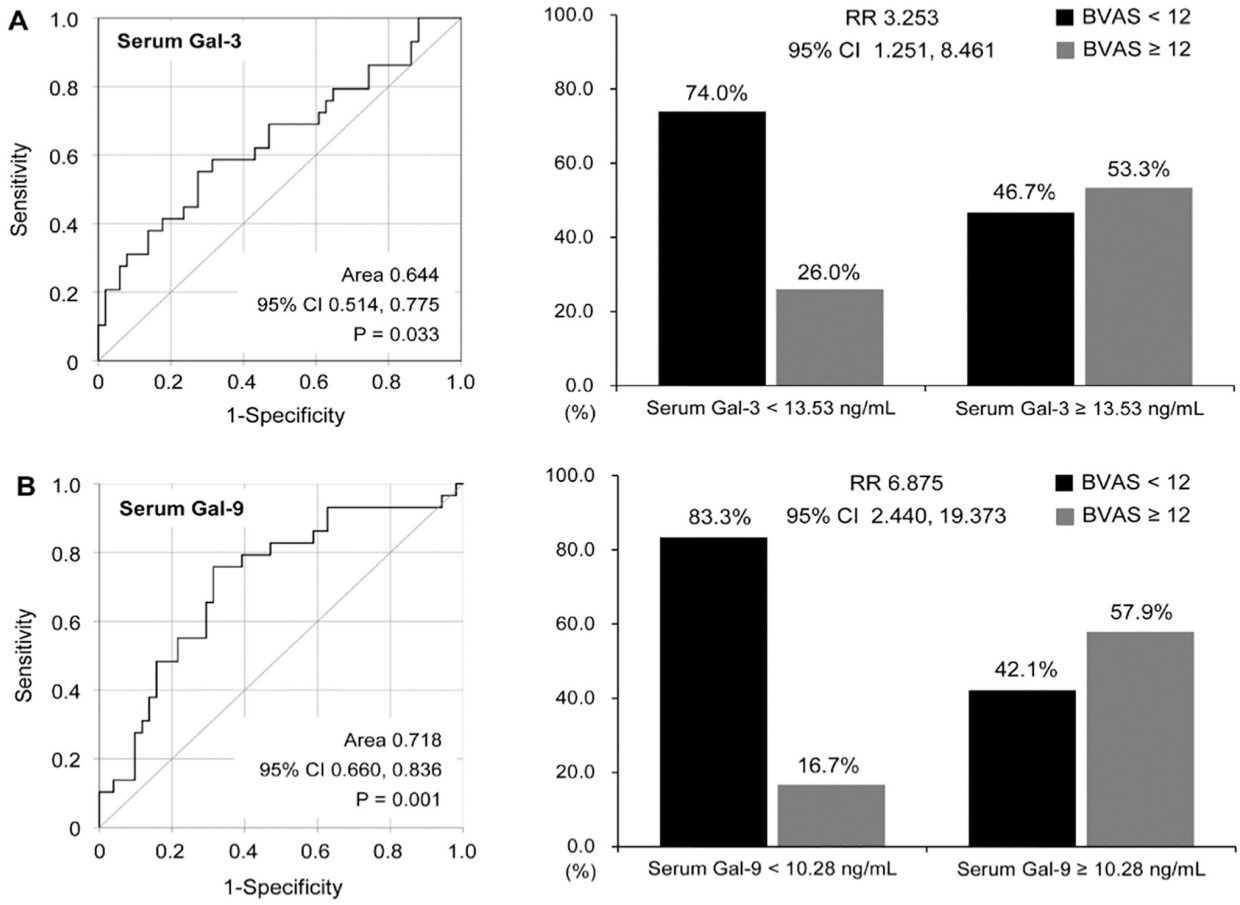

Fig. 1. Cut-off of serum Gal-3 and Gal-9 levels for high activity of AAV.
Patients with the absolute cut-off value of (**A**) serum Gal-3 ≥13.53 ng/mL and (**B**) serum Gal-9 ≥10.28 ng/mL had a significantly higher risk of having high activity than those without.
Gal: galectin; AAV: ANCA-associated vasculitis; ANCA: antineutrophil cytoplasmic antibody; RR: relative risk; BVAS: Birmingham vasculitis activity score.

Table IV. Logistic regression analyses based on high activity of AAV.

Variables	Univariable			Multivariable (serum galectin-3 and -9)			Multivariable (Cut-off of serum galectin-3 and -9)		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Age	1.019	0.986, 1.054	0.265						
Male sex	0.691	0.275, 1.735	0.431						
MPO-ANCA (or P-ANCA) positivity	2.384	0.864, 6.580	0.093						
PR3-ANCA (or C-ANCA) positivity	0.865	0.199, 3.755	0.847						
ESR	1.021	1.007, 1.035	0.004	1.018	0.996, 1.041	0.114			
CRP	1.017	1.003, 1.030	0.015	1.005	0.986, 1.024	0.632			
Galectin-3	1.170	1.032, 1.327	0.014	1.138	0.973, 1.332	0.107			
Galectin-9	1.127	1.039, 1.223	0.004	1.081	0.977, 1.195	0.130			
ESR	1.021	1.007, 1.035	0.004			1.021	0.997, 1.045	0.089	
CRP	1.017	1.003, 1.030	0.015			1.004	0.984, 1.023	0.723	
Galectin-3 ≥13.53 ng/mL	3.253	1.251, 8.461	0.016			2.481	0.767, 8.028	0.129	
Galectin-9 ≥10.28 ng/mL	6.875	2.440, 19.373	<0.001			5.303	1.643, 17.114	0.005	

AAV: ANCA-associated vasculitis; ANCA: antineutrophil cytoplasmic antibody; MPO: myeloperoxidase; P: perinuclear; PR3: proteinase 3; C: cytoplasmic; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

We questioned why serum Gal-1, Gal-3, and Gal-9 levels differ solely depending on the presence or absence of renal manifestation in AAV patients. A previous study demonstrated that T_H17/Treg cell imbalance is associated with renal involvement and disease remission in AAV patients (20). Applying this result to AAV patients, we hypothesised that given the roles of galectins, the production and secretion of Gal-1, Gal-3, and Gal-9 might be promoted to reduce the inflammatory burden in the kidneys by suppressing T_H17 cell activation and IL-17 production, thereby

Table V. Comparison of serum levels of galectins and cytokines according to the presence of MPO-ANCA (or P-ANCA) and PR3-ANCA (C-ANCA) or renal involvement.

Variables	MPO-ANCA (or P-ANCA) negative	MPO-ANCA (or P-ANCA) positive	*p*-value
Galectin-1	35.0 (24.3)	40.0 (25.7)	0.032
Galectin-3	10.7 (5.3)	13.3 (5.7)	0.005
Galectin-9	8.4 (6.9)	11.1 (10.4)	0.047
Interleukin-6	2.7 (3.9)	3.1 (7.0)	0.158
Interleukin-8	29.6 (79.9)	20.0 (83.8)	0.832
	PR3-ANCA (or C-ANCA) negative	PR3-ANCA (or C-ANCA) positive	*p*-value
Galectin-1	39.3 (22.5)	24.8 (25.6)	0.058
Galectin-3	13.1 (5.7)	8.6 (4.6)	0.004
Galectin-9	11.0 (9.2)	5.6 (7.2)	0.026
Interleukin-6	3.1 (5.0)	2.3 (4.3)	0.207
Interleukin-8	22.3 (80.1)	9.2 (71.0)	0.056
Variables	Absence of renal manifestation	Presence of renal manifestation	*p*-value
Galectin-1	29.7 (18.0)	43.8 (20.5)	<0.001
Galectin-3	10.6 (4.6)	13.6 (5.9)	<0.001
Galectin-9	7.1 (4.5)	13.7 (9.4)	<0.001
Interleukin-6	2.7 (5.0)	3.6 (5.3)	0.054
Interleukin-8	26.0 (121.0)	19.3 (58.9)	0.328

Values are expressed as a median (interquartile range, IQR).
MPO: myeloperoxidase; ANCA: antineutrophil cytoplasmic antibody; P: perinuclear; PR3: proteinase 3; C: cytoplasmic.

promoting Treg cell function. Furthermore, another previous study reported that serum Gal-9 level is significantly elevated only in lupus patients with active renal involvement among various organs, except for increased cerebrospinal fluid level of Gal-9 in patients with neuropsychiatric lupus (10). Accordingly, we hypothesised that the degree of inflammation in the kidney attributes to the elevation of serum Gal-9 levels, which could potentially counteract active inflammation. In the upcoming future, examining the association between serum galectin levels and histological kidney inflammation and damage in patients with AAV will be crucial to prove the aforementioned hypotheses.

Among all AAV subtypes, serum Gal-3 and Gal-9 levels in patients with EGPA were significantly lower than those in patients with MPA and GPA (p=0.037 and p=0.020), respectively (Suppl. Fig. S3). Therefore, we investigated and validated the association between high activities with both serum levels of Gal-3 and Gal-9 in 65 patients with MPA and GPA. When logistic regression analyses were conducted in these patients, serum Gal-9 ≥10.28 ng/mL was independently associated with high activity (Suppl. Table S2), in line with the finding observed in the total patients. Therefore, serum Gal-9 level was revealed to be a useful biomarker in assessing the disease activity not only in patients with all AAV subtypes, but also in subgroups of patients with MPA and GPA.

To our knowledge, this study is the first to identify that serum Gal-9 could be a potential biomarker of AAV among various galectins. However, this study has several limitations. First, the number of included patients was relatively small and the levels of galectins were not assessed serially. Second, we did not compare the levels of galectins with those in healthy controls. Third, although a significant relationship between serum Gal-9 level and disease activity of AAV was demonstrated, a direct association with its pathogenesis could not elucidated. Therefore, we believe that further research is necessary to understand the role of serum Gal-9 levels in AAV patients precisely.

Conclusion

In conclusion, this study demonstrated that serum Gal-9 levels were associated with BVAS in patients with AAV. Therefore, we suggest that serum Gal-9 level could be a potential disease activity biomarker in AAV. Additional researches are required to investigate the clinical utility of serum Gal-9 as a reliable surrogate marker of AAV disease activity.

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