Soluble Lectin-Like Oxidized Low-Density Lipoprotein Receptor 1 Is Inversely Correlated with the Activity of ANCA-Associated Vasculitis

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Lectin-like oxidized low-density lipoprotein (LDL) receptor 1 (LOX1) binds to oxidized LDL, which is associated with inflammation in various vascular disorders. Here, we aimed to investigate the potential of soluble LOX1 (sLOX1) as an indicator of antineutrophil cytoplasmic antibody-associated vasculitis (AAV) activity. Serum levels of sLOX1 in frozen samples from patients with AAV enrolled in a prospective observational cohort study at the Severance Hospital were measured using enzyme-linked immunosorbent assay. Clinical and laboratory data were collected on the date when the blood sampling was performed. The association between sLOX1 and clinical and laboratory data was assessed using Pearson's correlation analysis. The median age of the recruited 79 patients was 62.0 years, and 27 (34.2%) patients were men. The median Birmingham vasculitis activity score (BVAS), five-factor score, vasculitis damage index, and sLOX1 level were 6, 1, 3, and 911.9 pg/mL, respectively. Correlation analysis based on BVAS revealed that sLOX1 and total cholesterol were significantly inversely correlated with BVAS (r=-0.224, p=0.047 and r=-0.424, p<0.001, respectively). No significant correlations were observed between continuous variables and sLOX1 except for BVAS, although total cholesterol tended to correlate with sLOX1 (r=0.190, p=0.093). Additionally, sLOX1 was not influenced by sex, hypertension, diabetes mellitus, or the presence of pulmonary, cardiovascular, and renal involvement of AAV. In summary, sLOX1 was inversely correlated with BVAS in AAV patients, which is different from other vascular diseases or inflammatory diseases.

Key Words: Antineutrophil cytoplasmic antibody, vasculitis, lectin-like oxidized low-density lipoprotein receptor 1, activity

Lectin-like oxidized low-density lipoprotein (LDL) receptor 1 (LOX1) is a receptor that recognizes oxidized LDL (oxLDL). LOX1 is expressed in various cells, such as endothelial cells and macrophages.[1] OxLDL binding to LOX1 results in induction of endothelial dysfunction, monocyte infiltration, and LOX1 activation via intracellular signaling and inflammatory substances.

Specifically, oxLDL increases reactive oxygen species synthesis, LOX1 expression, and foam cell formation in macrophages through LOX1 while inhibiting macrophage migration, thereby leading to accelerated atherosclerosis.[2] Proinflammatory cytokines may enhance the proteolytic function of metalloproteinases such as ADAM10 and 17, which, in turn, may augment the cleavage of the extracellular domain of LOX1 and increase the serum soluble LOX1 (sLOX1) levels.[3] Furthermore, oxLDL was reported to enhance sLOX1 release in human umbilical vein endothelial cells in vitro.[4] Therefore, sLOX1 may reflect not only an increase in the intracellular LOX1 expression by oxLDL, but also a proinflammatory status. Based on these findings, even though sLOX1 is mainly considered to be a predictive biomarker for estimating the occurrence of cardiovascular diseases in various disorders, sLOX1 was also found to be associated with the disease activity of rheumatoid arthritis (RA), which is a typical autoimmune disease affecting the joints.[5]

Antineutrophil cytoplasmic antibody (ANCA)-associated

vasculitis (AAV) is a small vessel vasculitides characterized by inflammation in intraparenchymal capillaries, arterioles, and venules. Based on clinical and pathological findings, AAV is categorized into three variants: microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), and eosinophilic granulomatosis with polyangiitis (EGPA).[6,7] AAV pathogenesis is similar to the pathogenesis of atherosclerosis—mediated through oxLDL and LOX1—which involves endothelial dysfunction and macrophage activation via primed and activated neutrophils and circulating ANCA.[8] Thus, theoretically, sLOX1 is expected to reflect the activity of AAV; however, this has not been investigated. Therefore, in this study, we evaluated the potential of sLOX1 as a biomarker to reflect the activity of AAV.

Seventy-nine patients with AAV, who had been enrolled in the Severance Hospital ANCA associated VasculitidEs (SHAVE) cohort study from November 2016 to April 2019, were included in this study. The SHAVE cohort is a prospective observational cohort of Korean patients with AAV. Blood samples of the patients are collected at a regular basis of every 3 to 6 months simultaneously using clinical and laboratory data, and patients' sera are stored at -80°C upon isolation. Written informed consents were obtained from patients when the blood sampling was performed. All patients enrolled in the cohort were diagnosed as AAV at Severance Hospital, and met the 2007 European Medicines Agency algorithms for defining AAV and polyarteritis nodosa[6] as well as the 2012 Chapel Hill Consensus Conferences Nomenclature of Vasculitis.[7] Here, we used the patients' clinical and laboratory data, including demographic data, AAV variants, ANCA positivity, clinical manifestations, vasculitic indices, comorbidities, and laboratory results, on the date when blood sampling was performed. Meanwhile, patients with serious medical conditions other than AAV, such as severe infections and malignancies, were excluded from this study. Vasculitic indices for AAV included Birmingham vasculitis activity score (BVAS),[9] five-factor score (FFS) as proposed in 2009,[10] vasculitis damage index (VDI),[11] and the Korean version of the short form 36-item Health Survey (SF-36) as a functional index in AAV patients. SF-36 was scored and represented as both mental component score and physical component score.[12] We evenly applied BVAS to MPA, GPA, and EGPA patients to normalize the scoring system, as BVAS for GPA has a different weight-system compared with BVAS. Medications that were prescribed to the patients for the treatment of dyslipidemia and AAV were also investigated by searching the electronic medical records of patients.

Serum sLOX1 levels were measured in stored samples with enzyme-linked immunosorbent assay kits (ab212161; Abcam, Cambridge, UK) in accordance with the manufacturer's instructions. In addition, sera of 37 healthy controls were used to compare the level of sLOX1 in AAV and controls. This study was performed in accordance with the principles set by the Declaration of Helsinki and its later amendments, and was approved by the Institutional Review Board of Severance Hos-

**Table 1.** Clinical Characteristics of Patients

Variables	Values
Demographic data	
Age (yr)	62.0 (21.0)
Male sex	27 (34.2)
Disease duration (month)	2.3 (21.1)
Variants of AAV	
MPA	42 (53.2)
GPA	21 (26.5)
EGPA	16 (20.3)
ANCA	
MPO-ANCA (or P-ANCA) positivity	45 (57.0)
PR3-ANCA (or C-ANCA) positivity	7 (8.9)
Clinical manifestations	
General	26 (32.9)
Cutaneous	9 (11.4)
Mucous membranes/eyes	5 (6.3)
Ear Nose Throat	35 (44.3)
Pulmonary	50 (63.3)
Cardiovascular	4 (5.1)
Gastrointestinal	0 (0.0)
Renal	40 (50.6)
Nervous	17 (21.5)
Vasculitic indices	
BVAS	6 (10)
FFS	1 (1)
VDI	3 (2)
SF36-PCS	48.4 (35.6)
SF36-MCS	55.9 (33.8)
Comorbidities	
Hypertension	19 (24.1)
Diabetes mellitus	14 (17.7)
Laboratory results	
White blood cell count (/mm³)	7700.0 (4620.0)
Haemoglobin (g/dL)	11.8 (3.8)
Platelet (×1000/mm³)	253.0 (134.0)
Fasting glucose (mg/dL)	101.0 (35.0)
Blood urea nitrogen (mg/dL)	18.9 (16.5)
Creatinine (mg/dL)	0.9 (1.6)
Total protein (g/dL)	6.4 (0.9)
Serum albumin (g/dL)	3.7 (0.9)
Aspartate aminotransferase (IU/L)	18.0 (9.0)
Alanine aminotransferase (IU/L)	18.0 (13.0)
ESR (mm/hr)	37.0 (42.0)
CRP (mg/L)	2.3 (14.6)
Total cholesterol (mg/dL)	188.6 (55.9)
sLOX1 (pg/mL)	911.9 (1372.1)

AAV, antineutrophil cytoplasmic antibody-associated vasculitis; MPA, microscopic polyangiitis; GPA, granulomatosis with polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis; ANCA, antineutrophil cytoplasmic antibody; MPO, myeloperoxidase; P, perinuclear; PR3, proteinase 3; C, cytoplasmic; BVAS, birmingham vasculitis activity score; FFS, five-factor score; VDI, vasculitis damage index; SF36-PCS, short form 36-item health survey physical component score; SF36-MCS, short form 36-item health survey mental component score; ESR, erythrocyte sedimentation rate; CRP, c-reactive protein; sLOX1, soluble lectin-like oxidized low-density lipoprotein receptor 1.
Values are expressed as a median (interquartile range) or number (%).

pital (4-2016-0901). All statistical analyses were conducted using SPSS software (version 23 for Windows; IBM Corp., Armonk, NY, USA). Continuous variables were expressed as a median (interquartile range), and categorical variables were expressed as number (percentage). The correlation coefficient of continuous variables was obtained using the Pearson's correlation coefficient analysis. Significant differences in continuous variables between the two groups were compared using the Mann–Whitney U and Kruskal-Wallis test. p-values< 0.05 were considered statistically significant.

The median age of patients was 62.0 years, and 27 (34.2%) patients were men. The median BVAS, FFS, and VDI were 6, 1, and 3, respectively. Clinical and laboratory data are presented in Table 1. The median serum sLOX1 was 911.9 pg/mL, and patients with AAV had significantly lower sLOX1 level in the sera compared to healthy controls (p<0.001) (Supplementary Fig. 1, only online). Correlation analysis based on BVAS revealed that sLOX1 and total cholesterol were significantly inversely correlated with BVAS (r=-0.224, p=0.047 and r=-0.424, p<0.001), whereas FFS, VDI, erythrocyte sedimentation rate,
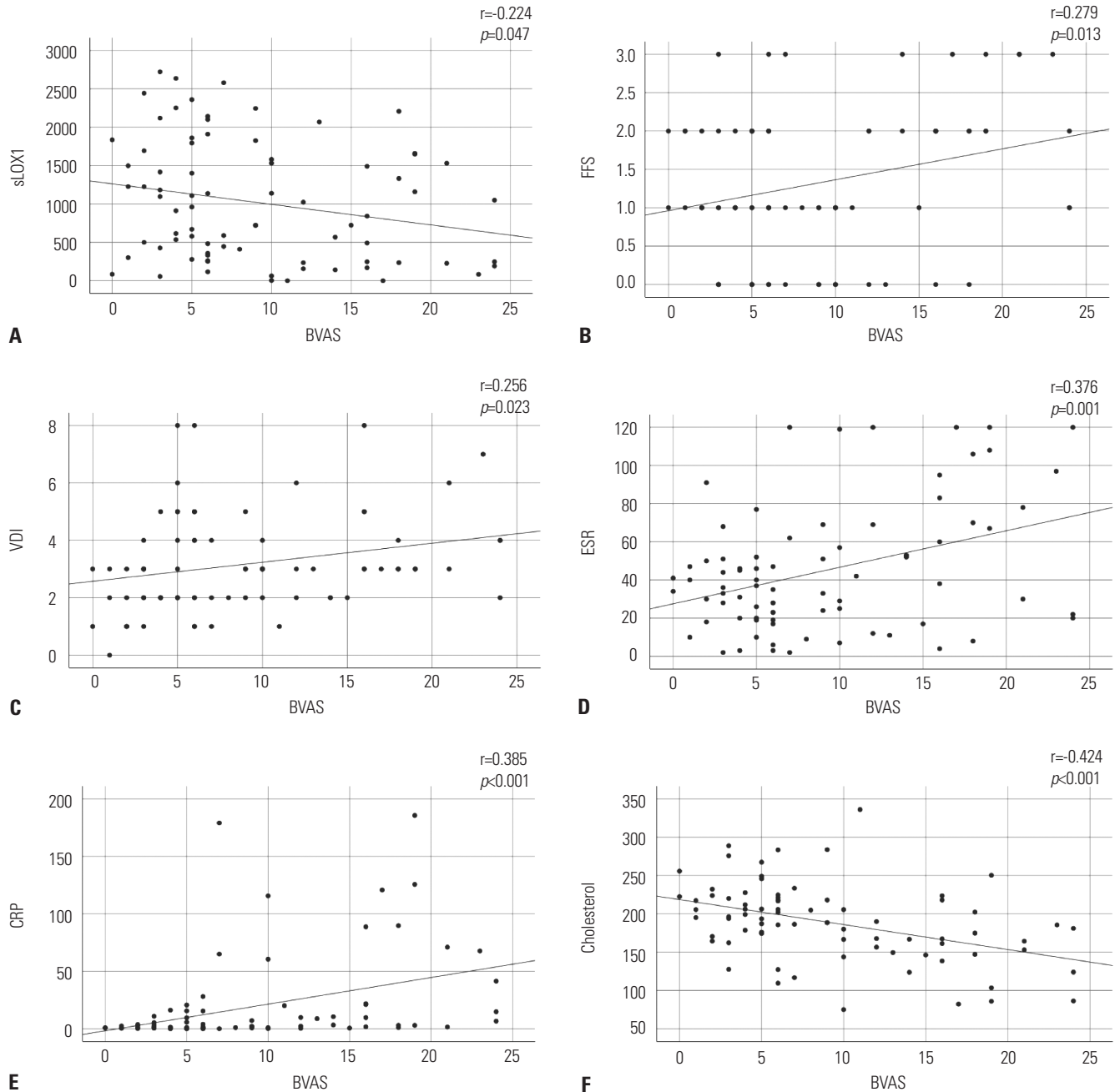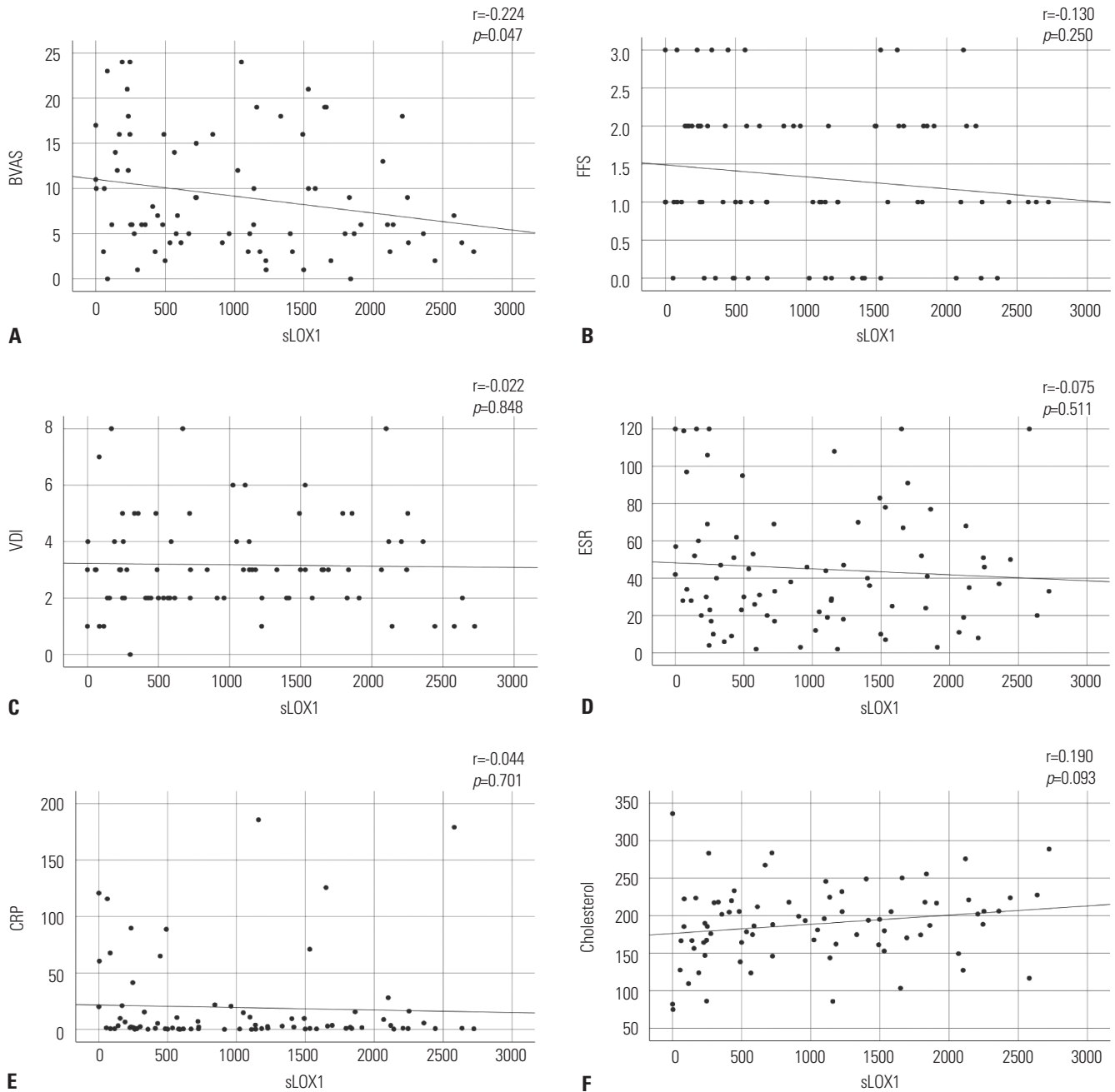

Fig. 1. Correlation between BVAS and continuous variables. The association between BVAS and (A) sLOX1, (B) FFS, (C) VDI, (D) ESR, (E) CRP, and (F) cholesterol level was assessed using Pearson's correlation analysis. BVAS, birmingham vasculitis activity score; sLOX1, soluble lectin-like oxidized low-density lipoprotein receptor 1; FFS, five-factor score; VDI, vasculitis damage index; ESR, erythrocyte sedimentation rate; CRP, c-reactive protein.

and C-reactive protein were significantly positively correlated with BVAS (Fig. 1). However, correlation analysis based on sLOX1 did not reveal any significant correlations between these variables and sLOX1, except for BVAS, although total cholesterol tended to correlate with sLOX1 (r=0.190, p=0.093) (Fig. 2). Furthermore, no difference was found in sLOX1 level regarding the use of medications and disease variants (Supplementary Table 1 and Supplementary Fig. 2, only online). In addition, we found that the serum sLOX1 levels in these patients were not influenced by sex, hypertension, diabetes mellitus, or by the presence of pulmonary, cardiovascular, and renal involvement of AAV (Supplementary Fig. 3, only online).

It has been demonstrated that inflammatory cytokines could increase sLOX1 by enhancing the enzymatic function of ADAM10 and 17, which releases sLOX1 as a result of proteolytic cleavage.[3] During the pathogenesis of AAV, the release of inflammatory cytokines from macrophages could promote ANCA production as well as neutrophil priming and activation, thereby triggering and exacerbating AAV.[8] Therefore, in theory, BVAS—reflecting the activity of AAV—should be posi-

Fig. 2. Correlation between sLOX1 and continuous variables. The association between sLOX1 and (A) BVAS, (B) FFS, (C) VDI, (D) ESR, (E) CRP, and (F) cholesterol level was assessed using Pearson's correlation analysis. sLOX1, soluble lectin-like oxidized low-density lipoprotein receptor 1; BVAS, birmingham vasculitis activity score; FFS, five-factor score; VDI, vasculitis damage index; ESR, erythrocyte sedimentation rate; CRP, c-reactive protein.

tively correlated with sLOX1; however, to our surprise, BVAS was significantly inversely correlated with sLOX1.

A previous study has shown that serum level of sLOX1 and oxLDL is significantly elevated in patients with active Behçet's disease and systemic lupus erythematosus compared to healthy controls.[13] In the present study, we did not measure the serum oxLDL, but measured the total serum cholesterol instead, which might be proportional to serum oxLDL. We found that a negative correlation between disease activity and sLOX1 was present; and when we compared sLOX1 with healthy controls, it was shown that the level of sLOX1 was significantly lower in AAV patients compared to healthy controls. Although the precise cause of this observation remains unknown, the decrease in total cholesterol could be attributed to this result. Total cholesterol level could decrease in chronic inflammatory conditions; in this context, it has been reported that patients with RA have lower total cholesterol level compared to the general population.[14] Interestingly, a recent publication by Wallace, et al.[15] also reported that total cholesterol levels were suppressed prior to treatment and elevated after 6 months of treatment in AAV patients, suggesting that lower total cholesterol levels are indicative of higher inflammation and disease activity. In fact, we also found that when the activity of AAV (BVAS) increases, a proportional decrease of total cholesterol level is observed (r= -0.424). Owing to the fact that the production of sLOX1 is mainly dependent on the increased concentration of oxLDL, which was shown in an in vitro experiment,[4] it is possible that sLOX1 is decreased in AAV as a consequence of decreased cholesterol levels. However, a report by Petermann Smits, et al.[16] has shown that neither total cholesterol nor LDL levels were significantly different between AAV patients and controls. Given that the association between BVAS and total cholesterol level in AAV still remains controversial, further studies are required to elucidate whether cholesterol profiles in the blood are correlated with the disease activity of AAV.

To the best of our knowledge, the strength of this study is that it is the first to investigate the potential of sLOX1 as a biomarker to assess the activity of AAV. However, our study had several limitations. First, although this was a prospective cohort study, we were not able to provide longitudinal information on sLOX1 in AAV patients. Second, the number of patients included in this study was not large enough to represent all AAV patients. Third, our data cannot provide any mechanistic investigation into how sLOX1 could be directly linked to disease activity in AAV. Fourth, we were not able to evaluate LOX1 expression in the affected tissues, which may provide more reliable information regarding the role of LOX1 in AAV. Finally, we could not enroll patients from other regions as a validation cohort to verify the findings of our study.

In conclusion, our study demonstrates that sLOX1 is inversely correlated with BVAS in AAV patients, which is contrary to other vascular diseases or inflammatory diseases. Additional studies are warranted to elucidate the clinical implication of sLOX1 in patients with AAV.

AUTHOR CONTRIBUTIONS

Conceptualization: Sung Soo Ahn and Sang-Won Lee. Data curation: Taejun Yoon and Sung Soo Ahn. Formal analysis: Taejun Yoon and Sung Soo Ahn. Funding acquisition: Sang-Won Lee. Investigation: Taejun Yoon and Sung Soo Ahn. Methodology: Taejun Yoon and Sung Soo Ahn. Project administration: Taejun Yoon, Sung Soo Ahn, and Sang-Won Lee. Resources: Jason Jungsik Song, Yong-Beom Park, and Sang-Won Lee. Software: Sung Soo Ahn. Supervision: Jason Jungsik Song, Yong-Beom Park, and Sang-Won Lee. Validation: Taejun Yoon and Sung Soo Ahn. Visualization: Taejun Yoon and Sung Soo Ahn. Writing—original draft: Taejun Yoon and Sung Soo Ahn. Writing—review & editing: all authors. Approval of final manuscript: all authors.

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