

Association between follistatin-related protein 1 and the functional status of patients with anti-neutrophil cytoplasmic antibody-associated vasculitis

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Abstract

Background: Follistatin-like 1 (FSTL1) plays both pro-inflammatory and anti-inflammatory roles in the inflammatory processes. We investigated whether serum FSTL1 could predict the current anti-neutrophil cytoplasmic antibody-associated vasculitis (AAV)-specific indices.

Methods: We randomly selected 74 patients with AAV from a prospective and observational cohort of Korean patients with AAV. Clinical and laboratory data and AAV-specific indices were recorded. FSTL1 concentration was determined using the stored sera. The lowest tertile of the short-form 36-item health survey (SF-36) was defined as the current low SF-36. The cutoffs of serum FSTL1 for the current low SF-36 physical component summary (PCS) and SF-36 mental component summary (MCS) were extrapolated by the receiver operator characteristic curve.

Results: The median age was 62.5 years (55.4% were women). Serum FSTL1 was significantly correlated with SF-36 PCS ($r = -0.374$), SF-36 MCS ($r = -0.377$), and C-reactive protein (CRP) ($r = 0.307$), but not with Birmingham vasculitis activity score (BVAS). In the multivariable linear regression analyses, BVAS, CRP, and serum FSTL1 were independently associated with the current SF-36 PCS ($\beta = -0.255$, $\beta = -0.430$, and $\beta = -0.266$, respectively) and the current SF-36 MCS ($\beta = -0.234$, $\beta = -0.229$, and $\beta = -0.296$, respectively). Patients with serum FSTL1 ≥ 779.8 pg/mL and those with serum FSTL1 ≥ 841.6 pg/mL exhibited a significantly higher risk of having the current low SF-36 PCS and SF-36 MCS than those without (relative risk 7.583 and 6.200, respectively).

Conclusion: Serum FSTL1 could predict the current functional status in AAV patients.

Keywords: Anti-neutrophil cytoplasmic antibody; Follistatin-like 1; Functional status; Vasculitis

Introduction

Systemic vasculitides are categorized by the size of affected vessels into large, medium, small, and variable vessel vasculitis. According to the presence of immune complex in tissue pathology, small vessel vasculitis (SVV) is further divided into immune complex SVV and anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV); AAV affects capillaries, venules, arterioles, and small arteries and is characterized by necrotizing vasculitis with few or no deposit of immune complexes based on the 2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides.^[1] Furthermore, AAV is composed of three subtypes, namely microscopic

polyangiitis (MPA), granulomatosis with polyangiitis (GPA), and eosinophilic GPA (EGPA).^[1,2]

In our cohort of AAV patients, we usually collect AAV-specific indices at every 3- to 6-month visit: Birmingham vasculitis activity score (BVAS, version 3) and five-factor score (FFS) are calculated for assessing the current activity and the prognosis, respectively;^[3,4] vasculitis damage index (VDI) is evaluated for estimating the current extent of organ damage;^[5] and the Korean version of the short-form 36-item health survey physical and mental component summaries (SF-36 PCS and SF-36 MCS) is collected for predicting the current functional status.^[6] In particular, BVAS (version 3) is evenly applied to MPA, GPA, and

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EGPA patients to unify the scoring system. The forms of BVAS, FFS, and VDI are primarily completed by physicians, whereas SF-36 PCS and SF-36 MCS are mainly recorded by patients. To date, several studies on the discovery of biomarkers predicting AAV-specific indices, especially those related to the pathogenesis of AAV have been conducted. Biomarkers can predict AAV-specific indices quickly and easily even in difficult situations such as poor mental or physical states and a new concept of AAV pathogenesis can also be established by them. Therefore, the demand for biomarkers is constantly increasing.

Follistatin-like 1 (FSTL1), a bone morphogenetic protein-4 binding protein, is known to be associated with the development of several organs.^[7,8] In addition, FSTL1 has been reported to play a dual and opposing regulatory role in the inflammatory processes. It plays a pro-inflammatory role in rheumatoid arthritis synoviocytes through mitogen-activated protein kinase, Janus kinase/signal transducers and activators of transcription, and nuclear factor-kappa B (NF- κ B) signaling^[9]; conversely, it has a protective role in the heart through alleviating ischemia and reperfusion injury in cardiomyocytes.^[10]

A previous study on the role of FSTL1 in systemic vasculitis reported an association between plasma FSTL1 and coronary artery aneurysm in patients with Kawasaki disease.^[11] Given the earlier findings and the role of FSTL1 in the inflammatory processes, it can be theoretically assumed that serum FSTL1 may predict the current AAV-specific indices. However, there is no study regarding the clinical implication of serum FSTL1 for predicting those indices to date. Therefore, in this study, we recorded the current BVAS, FFS, VDI, SF-36 PCS, and SF-36 MCS at the time of blood sampling, and measured FSTL1 in the stored serum. Furthermore, we investigated whether serum FSTL1 could predict the current AAV-specific indices in patients who were recently diagnosed with AAV.

Methods

Ethical approval

This study was approved by the Institutional Review Board of Severance Hospital (No. 4-2016-0901) and written informed consent was obtained from the patients at the time of blood sampling.

Patients

We randomly selected 74 patients with AAV, who had been enrolled in the Severance hospital ANCA-associated vasculitides cohort, a prospective and observational cohort of Korean patients with AAV, from November 2016 to January 2020, and included them in this study. All patients were initially diagnosed with AAV at the Division of Rheumatology, Department of Internal Medicine, Yonsei University College of Medicine and Severance Hospital and they met both classification criteria, namely the 2007 European Medicines Agency Algorithms for AAV and Polyarteritis Nodosa and the 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides.^[1,2] AAV patients with serious medical

conditions, such as serious infections, malignancies, or other accompanying systemic vasculitides, were excluded from this study.

Clinical and laboratory data

All clinical data, including age, gender, AAV subtypes, and clinical manifestations based on BVAS and laboratory data, were recorded. AAV-specific indices including BVAS, FFS, VDI, SF-36 PCS, and SF-36 MCS were also recorded. ANCA was measured on the same day. Acute-phase reactants, namely the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level, were also measured. Myeloperoxidase (MPO)-ANCA and proteinase 3 (PR3)-ANCA were measured using the novel anchor-coated highly sensitive Phadia ELiA (Thermo Fisher Scientific/Phadia, Freiburg, Germany) and human native antigens, on the Phadia250 analyzer (Thermo Fisher Scientific/Phadia). In this study, 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 by an indirect immunofluorescence assay, they were considered to have MPO-ANCA or PR3-ANCA when AAV was strongly suspected based on the clinical and laboratory features.^[12] The currently administered AAV-related medications were also reviewed.

Blood samples and measurement of serum FSTL1

On the same visit day, whole blood was obtained from each AAV patients on patients' consent. Sera is immediately isolated from whole blood and stored at -80°C . FSTL1 concentration in stored sera was determined using enzyme-linked immunosorbent assay kits (Abcam, Cambridge, UK) according to the manufacturer's instructions. The sensitivity was <10 pg/mL and intra- and inter-assay coefficients of variation were 4.2% to 6.4% and 6.4% to 7.8%, respectively.

Definition of low SF-36

We defined the lowest tertile of SF-36 PCS and SF-36 MCS as the current low SF-36 PCS and SF-36 MCS, respectively. The cutoff values (the upper limit of the lowest tertile) of the current low SF-36 PCS and SF-36 MCS were determined as 38.4 and 46.8, respectively.

Statistical analyses

All statistical analyses were performed using SPSS software (version 25 for Windows; IBM Corp., Armonk, NY, USA). Continuous variables are expressed as medians with interquartile range (IQR), whereas categorical variables are expressed as numbers (percentage). The standardized correlation coefficient was obtained by the multivariable linear regression analysis using variables with statistical significance in the univariable linear regression analysis. The optimal cutoffs of serum FSTL1 for the current low SF-36 PCS and SF-36 MCS were extrapolated by conducting the receiver operator characteristic (ROC) curve and selecting the maximized sum of sensitivity and specificity. The relative risk (RR) was analyzed using the contingency

tables and the chi-square test. Significant differences in serum FSTL1 between the presence and absence of each clinical manifestation based on BVAS items were compared using the Mann-Whitney test. *P* values < 0.05 were considered statistically significant.

Results

Characteristics

The median age of patients was 62.5 (IQR = 20.0) years, and 41 patients were women. Of 74 patients, 35 were classified as MPA, 24 with GPA, and 15 with EGPA. Forty-four patients were detected with MPO-ANCA (or P-ANCA), whereas only six were positive for PR3-ANCA (or C-ANCA). The most frequently affected organ was the lung (58.1%), followed by the kidney (52.7%) and the ear, nose, and throat (44.6%). The median SF-36 PCS, SF-MCS, BVAS, FFS, and VDI were 50.8, 54.2, 7.5, 1.0, and 3.0, respectively. The median ESR and CRP levels were 30.5 mm/h and 2.9 mg/L, respectively. The median serum FSTL1 was 879.1 pg/mL. At sampling, 62 patients (83.8%) received glucocorticoid and the most frequently administered immunosuppressive drug was azathioprine (39.2%) followed by cyclophosphamide (8.1%). Rituximab was provided to one patient (1.4%) [Table 1].

Correlation analysis

Serum FSTL1 did not seem to be affected by age. Among the current AAV-specific indices, serum FSTL1 was significantly correlated with SF-36 PCS ($r = -0.374$, $P = 0.001$) and SF-36 MCS ($r = -0.377$, $P = 0.001$); however, it was not correlated with BVAS, FFS, and VDI. Among the current acute-phase reactants, serum FSTL1 was positively correlated with CRP level ($r = 0.307$, $P = 0.008$); however, it was not correlated with ESR [Figure 1].

Linear regression analyses

Based on the current SF-36 PCS, in the univariable analysis, BVAS ($\beta = -0.357$), FFS ($\beta = -0.229$), ESR ($\beta = -0.272$), CRP ($\beta = -0.484$), and FSTL1 ($\beta = -0.374$) were significantly correlated with the current SF-36 PCS. Furthermore, in the multivariable analysis, BVAS ($\beta = -0.255$, $P = 0.024$), CRP ($\beta = -0.430$, $P = 0.005$), and serum FSTL1 ($\beta = -0.266$, $P = 0.012$) were independently associated with the current SF-36 PCS. In addition, based on the current SF-36 MCS, in the univariable analysis, MPO-ANCA (or P-ANCA) ($\beta = -0.258$), BVAS ($\beta = -0.327$), CRP ($\beta = -0.433$), and serum FSTL1 ($\beta = -0.377$) were significantly correlated with the current SF-36 MCS. Furthermore, in the multivariable analysis, BVAS ($\beta = -0.234$, $P = 0.031$), CRP ($\beta = -0.229$, $P = 0.048$), and serum FSTL1 ($\beta = -0.296$, $P = 0.006$) exhibited an independent association with the current SF-36 MCS [Table 2].

Cutoff value of serum FSTL1 for the current low SF-36

The optimal cutoff value of serum FSTL1 for predicting the current low SF-36 PCS (SF-36 PCS ≤ 38.4) was evaluated

Table 1: Characteristics of 74 AAV patients at blood sampling.

| Variables | Values |
|--|---------------|
| Demographic data | |
| Age (years) | 62.5 (20.0) |
| Female, <i>n</i> (%) | 41 (55.4) |
| AAV subtypes, <i>n</i> (%) | |
| MPA | 35 (47.3) |
| GPA | 24 (32.4) |
| EGPA | 15 (20.3) |
| ANCA positivity, <i>n</i> (%) | |
| MPO-ANCA (or P-ANCA) positivity | 44 (59.5) |
| PR3-ANCA (or C-ANCA) positivity | 6 (8.1) |
| Both ANCAs positivity | 3 (4.1) |
| ANCA negative | 27 (36.5) |
| Clinical manifestations based on BVAS items, <i>n</i> (%) | |
| Generalised symptoms | 24 (32.4) |
| Skin | 8 (10.8) |
| Mucous membrane and eyes | 2 (2.7) |
| Ear nose and throat | 33 (44.6) |
| Lungs | 43 (58.1) |
| Heart | 4 (5.4) |
| Gastrointestine | 1 (1.4) |
| Kidneys | 39 (52.7) |
| Nervous system | 20 (27.0) |
| AAV-specific indices | |
| SF-36 PCS | 50.8 (32.3) |
| SF-36 MCS | 54.2 (28.0) |
| BVAS | 7.5 (10.0) |
| FFS | 1.0 (1.0) |
| VDI | 3.0 (2.0) |
| Acute-phase reactants | |
| ESR (mm/h) | 30.5 (49.0) |
| CRP (mg/L) | 2.9 (12.2) |
| Serum FSTL1 (pg/mL) | 879.1 (685.7) |
| AAV-related medications currently administered, <i>n</i> (%) | |
| Glucocorticoid | 62 (83.8) |
| Cyclophosphamide | 6 (8.1) |
| Rituximab | 1 (1.4) |
| Azathioprine | 29 (39.2) |
| Mycophenolate mofetil | 1 (1.4) |
| Tacrolimus | 2 (2.7) |
| Methotrexate | 3 (4.1) |

Values are expressed as median (interquartile range [IQR]) or number (percentage). AAV: Anti-neutrophil cytoplasmic antibody-associated vasculitis; ANCA: Anti-neutrophil cytoplasmic antibody; BVAS: Birmingham vasculitis activity score; C: Cytoplasmic; CRP: C-reactive protein; EGPA: Eosinophilic GPA; ESR: Erythrocyte sedimentation rate; FFS: Five-factor score; FSTL1: Follistatin-related protein 1; GPA: Granulomatosis with polyangiitis; MCS: Mental component summary; MPA: Microscopic polyangiitis; MPO: Myeloperoxidase; P: Perinuclear; PCS: Physical component summary; PR3: Proteinase 3; SF-36: The short-form 36-item health survey; VDI: Vasculitis damage index.

as 779.8 pg/mL using the ROC curve (area 0.727, 95% confidence interval [CI] 0.608, 0.846). Patients with AAV were divided into two groups based on the cutoff value of serum FSTL1; patients with serum FSTL1 ≥ 779.8 pg/mL exhibited a significantly higher risk of having the current low SF-36 PCS than those with FSTL1 < 779.8 pg/mL (RR 7.583, 95% CI 2.004, 28.698) [Figure 2]. In addition, the

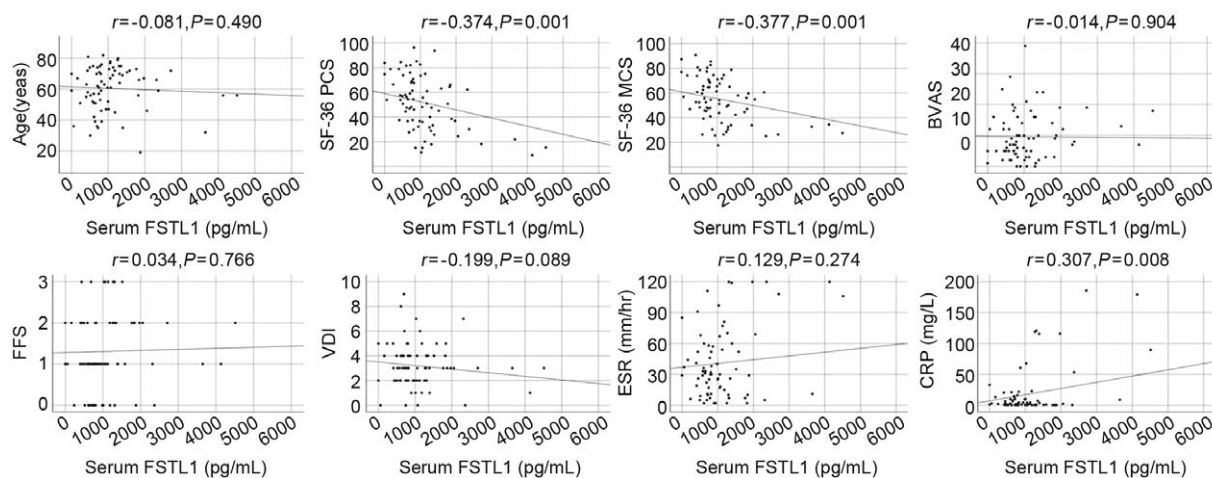


Figure 1: Serum FSTL1 was significantly correlated with SF-36 PCS, SF-36 MCS, and CRP, but not with age, BVAS, FFS, ESR, and VDI. BVAS: Birmingham vasculitis activity score; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; FFS: Five-factor score; FSTL1: Follistatin-like 1; MCS: Mental component summary; PCS: Physical component summary; SF-36: The Korean version of the short-form 36-item health survey; VDI: Vasculitis damage index.

Table 2: Linear regression analyses of variables based on SF-36 PCS and SF-36 MCS in AAV patients.

| Items | Univariable analysis | | | Multivariable analysis | | |
|----------------------|----------------------|-----------------|----------|------------------------|----------------|----------|
| | Standardized β | 95% CI | P values | Standardized β | 95% CI | P values |
| Based on SF-36 PCS | | | | | | |
| Age | -0.142 | -0.576, 0.139 | 0.227 | | | |
| MPO-ANCA (or P-ANCA) | -0.082 | -7.460, 3.577 | 0.485 | | | |
| PR3-ANCA (or C-ANCA) | 0.098 | -7.533, 18.460 | 0.405 | | | |
| BVAS | -0.357 | -1.620, -0.386 | 0.002 | -0.255 | -1.336, -0.095 | 0.024 |
| FFS | -0.229 | -10.631, -0.014 | 0.049 | -0.067 | -6.444, 3.346 | 0.530 |
| VDI | 0.006 | -2.766, 2.915 | 0.958 | | | |
| ESR | -0.272 | -0.308, -0.028 | 0.019 | 0.180 | -0.065, 0.288 | 0.212 |
| CRP | -0.484 | -0.372, -0.150 | <0.001 | -0.430 | -0.391, -0.073 | 0.005 |
| FSTL1 | -0.374 | -0.010, -0.003 | 0.001 | -0.266 | -0.008, -0.001 | 0.012 |
| Based on SF-36 MCS | | | | | | |
| Age | -0.162 | -0.501, 0.089 | 0.168 | | | |
| MPO-ANCA (or P-ANCA) | -0.258 | -9.468, -0.613 | 0.026 | -0.133 | -6.595, 1.419 | 0.202 |
| PR3-ANCA (or C-ANCA) | 0.076 | -7.261, 14.296 | 0.517 | | | |
| BVAS | -0.327 | -1.278, -0.245 | 0.004 | -0.234 | -1.039, -0.050 | 0.031 |
| FFS | -0.220 | -8.631, 0.177 | 0.060 | | | |
| VDI | -0.017 | -2.522, 2.180 | 0.885 | | | |
| ESR | -0.215 | -0.228, 0.007 | 0.066 | | | |
| CRP | -0.433 | -0.288, -0.099 | <0.001 | -0.229 | -0.204, -0.001 | 0.048 |
| FSTL1 | -0.377 | -0.009, -0.002 | 0.001 | -0.296 | -0.007, -0.001 | 0.006 |

AAV: Anti-neutrophil cytoplasmic antibody-associated vasculitis; ANCA: Anti-neutrophil cytoplasmic antibody; BVAS: Birmingham vasculitis activity score; C: Cytoplasmic; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; FFS: Five-factor score; FSTL1: Follistatin-related protein 1; MCS: Mental component summary; MPO: Myeloperoxidase; P: Perinuclear; PCS: Physical component summary; PR3: Proteinase 3; SF-36: The short-form 36-item health survey; VDI: Vasculitis damage index.

optimal cutoff value of serum FSTL1 for assessing the current low SF-36 MCS (SF-36 MCS ≤ 46.8) was determined as 841.6 pg/mL using the ROC curve (area 0.754, 95% CI 0.639, 0.869). Patients with AAV were divided into two according to the cutoff value of serum FSTL1; patients with serum FSTL1 ≥ 841.6 pg/mL exhibited a significantly higher risk of having the current low SF-36 MCS than those with FSTL1 < 841.6 pg/mL (RR 6.200, 95% CI 1.986, 19.360) [Figure 3].

Discussion

In this study, we investigated whether serum FSTL1 could predict the current AAV-specific indices including BVAS, FFS, VDI, SF-36 PCS, and SF-36 MCS, in 74 patients who were recently diagnosed with AAV; consequently, we had four observations. First, in the univariate correlation analysis, serum FSTL1 was significantly correlated with SF-36 PCS ($r = -0.374$), SF-36 MCS ($r = -0.377$), and CRP ($r = 0.307$), but it was not at all correlated with

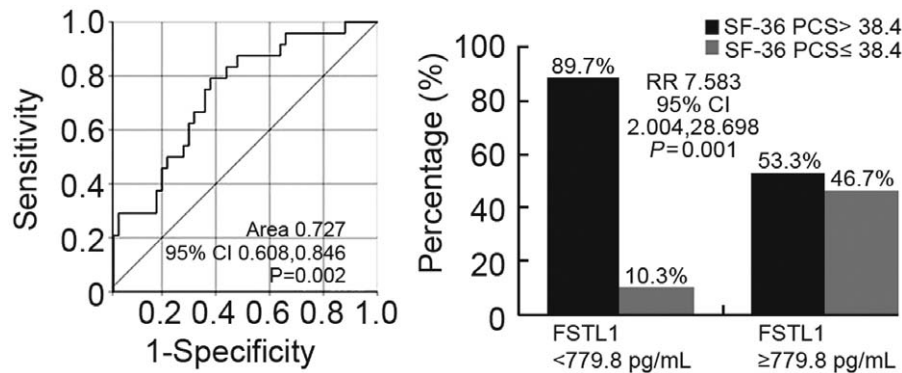


Figure 2: The cutoff value of serum FSTL1 for predicting the current low SF-36 PCS (SF-36 PCS ≤ 38.4) was set as 779.8 pg/mL. Patients with serum FSTL1 ≥ 779.8 pg/mL exhibited a significantly higher risk of having the current low SF-36 PCS than those with FSTL1 < 779.8 pg/mL. CI: Confidence interval; FSTL1: Follistatin-like 1; PCS: Physical component summary; RR: Relative risk; SF-36: The Korean version of the short-form 36-item health survey.

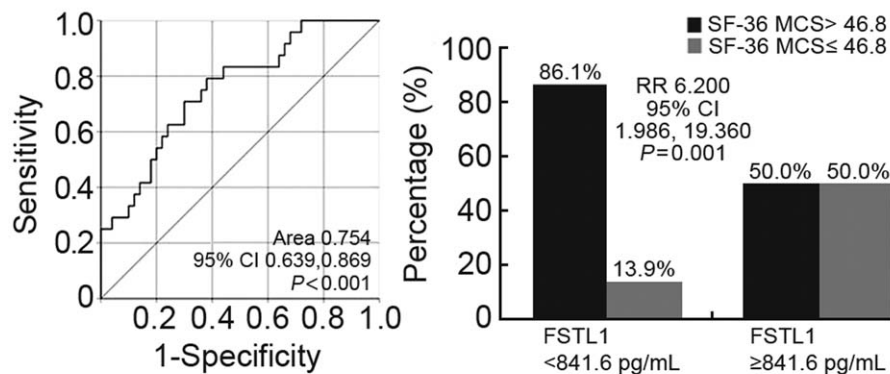


Figure 3: The cutoff value of serum FSTL1 for assessing the current low SF-36 MCS (SF-36 MCS ≤ 46.8) was set as 841.6 pg/mL. Patients with serum FSTL1 ≥ 841.6 pg/mL exhibited a significantly higher risk of having the current low SF-36 MCS than those with FSTL1 < 841.6 pg/mL. CI: Confidence interval; FSTL1: Follistatin-like 1; MCS: Mental component summary; RR: Relative risk; SF-36: The Korean version of the short-form 36-item health survey.

BVAS, FFS, and VDI. Second, in the multivariable linear regression analysis, serum FSTL1 was independently associated with both SF-36 PCS ($\beta = -0.266$) and SF-36 MCS ($\beta = -0.296$). Third, AAV patients with serum FSTL1 ≥ 779.8 pg/mL exhibited a significantly higher risk of having the current low SF-36 PCS than those with FSTL1 < 779.8 pg/mL (RR 7.583). Fourth, AAV patients with serum FSTL1 ≥ 841.6 pg/mL exhibited a significantly higher risk of having the current low SF-36 MCS than those with FSTL1 < 841.6 pg/mL (RR 6.200).

FSTL1 plays a dual and opposing regulatory role in the process of inflammation. As a pro-inflammatory role, it binds to pro-inflammatory receptors, such as toll-like receptor 4, accelerating the expression of pro-inflammatory cytokines through extracellular signal-regulated kinases 1/2, c-Jun N-terminal kinase, and NF- κ B pathways.^[9,13] Among increased pro-inflammatory cytokines, tumor necrosis factor- α , and interferon- γ directly augment the expression and secretion of FSTL1, whereas interleukin (IL)-1 β indirectly enhances its expression through the NF- κ B pathway, leading to the formation of a positive feedback loop.^[14] In addition, FSTL1 may enhance the proliferation and polarization of immune cells, particular-

ly, tissue macrophages, and aggravates inflammation.^[15] As an anti-inflammatory role, FSTL1 also binds to anti-inflammatory receptors, such as disco interacting protein 2 homolog A, resulting in downregulation of metalloproteinase 2, 3, and 13,^[9] as well as stimulation of the transforming growth factor β /Smad signaling pathway.^[16]

In this study, there was no significant correlation between serum FSTL1 and BVAS, although both serum FSTL1 and BVAS exhibited significant correlations with CRP (serum FSTL1 and CRP: $r = 0.307$, $P = 0.008$, and BVAS and CRP: $r = 0.336$, $P = 0.003$). We attempted to understand the reason through the following two different mechanisms. First, for the pro-inflammatory effect, serum FSTL1 may gradually increase BVAS and CRP production in the liver through increased IL-6.^[17] Second, for the anti-inflammatory effect, serum FSTL1 may gradually decrease BVAS but without affecting CRP production in the liver, through increased IL-6.^[16,18] In theory, serum FSTL1 is positively correlated with the current BVAS as well as CRP while exhibiting the pro-inflammatory effect. In contrast, serum FSTL1 is negatively correlated with the current BVAS but not with CRP, while exhibiting the anti-inflammatory effect. Therefore, the discrepancies in the

correlations of serum FSTL1 with BVAS and CRP can be explained by these dual effects of serum FSTL1 in the inflammatory processes.

We speculated on why serum FSTL1 was significantly associated with both SF-36 PCS and SF-36 MCS rather than BVAS and VDI. The approach to the answer should start from the temporal concept of the three indicators. A VDI reflects organ damage resulting from the inflammatory burden accumulated over a long period of time^[5]; it may not have shown a significant correlation with FSTL1 because the rate of fibrosis is higher than that of chronic inflammation.^[19] Since BVAS is associated with relatively the sub-acute or acute inflammatory processes,^[3] the aforementioned dual and opposing regulatory role of FSTL1 would interfere with and dilute the significant correlation between BVAS and FSTL1. On the other hand, since both SF-36 PCS and SF-36 MCS are relatively associated with the chronic inflammatory processes rather than acute or sub-acute ones,^[6] the pro-inflammatory role of FSTL1 may surpass its anti-inflammatory role, which may have enabled the correlation between serum FSTL1 and either SF-36 PCS, or SF-36 MCS.

This study describes a method to obtain the cutoff value of serum FSTL1 for predicting the current low SF-36 PCS and SF-36 MCS. Regarding the clinical significance of determining the cutoff value of serum FSTL1 for predicting the current low SF-36 PCS and SF-36 MCS, both are the most widely used self-reported indices for assessing health-related quality of life (HRQOL) as frequently as the health assessment questionnaire scores.^[6,20] Additionally, they are currently used for predicting the functional status, which is considered as an important therapeutic goal as predicting the current activity and organ damage in patients with AAV. Therefore, clinical trials enrolling AAV patients have included SF-36 PCS and SF-36 MCS as the essential indicators for determining the effectiveness of therapeutic drugs to date.^[21,22] Nevertheless, in a real clinical setting, it is not easy to complete the SF-36 form at every visit for the following individuals: elderly patients complaining of decreased vision, patients suffering from cognitive dysfunction and not accompanied by a caregiver, and patients treated in the intensive care unit due to serious organ damage. If there is an indicator that predicts the current low SF-36 PCS and SF-36 MCS, such as serum FSTL1, a qualitative evaluation, but not a quantitative evaluation of the functional status of AAV may be possible in these patients.

Although there was no significant correlation between serum FSTL1 and the current BVAS, we wonder whether serum FSTL1 may differ according to the presence of each clinical manifestation based on BVAS items,^[3] and compared serum FSTL1 in the presence and absence of each clinical manifestation. Among nine clinical manifestations based on BVAS items, serum FSTL1 tended to differ according to the manifestation of heart disease. Given that a previous study reported the predictive potential of plasma FSTL1 for coronary artery aneurysm in patients with Kawasaki disease, we compared the median serum FSTL1 between the two groups.^[11] However, the median serum FSTL1 was much lower in patients with the manifestation of heart disease than in patients without

the manifestation (655.2 *vs.* 963.0 pg/mL), although the difference was not statistically significant ($P = 0.090$). Furthermore, among four AAV patients with heart involvement, three presented pericarditis and one exhibited myocarditis without the evidence of coronary arterial involvement. Together, these results suggest that the clinical implication of FSTL1 in systemic vasculitides may vary depending on the size of the affected vessel and the entity of each vasculitis.^[11]

To the best of our knowledge, the clinical implications of serum FSTL1 in AAV have never been investigated previously. Thus, this is the first pilot study to reveal that serum FSTL1 was significantly and independently correlated with the current SF-36 PCS and SF-36 MCS, but not with BVAS, FFS, and VDI in AAV patients. Moreover, we described the method to obtain the cutoff values of serum FSTL1 for predicting the current low SF-36 PCS and SF-36 MCS (the lowest tertile of each SF-36) and reported the RR associated with more than one cutoff values of serum FSTL1 for predicting the current low SF-36.

However, our study has several limitations. The number of AAV patients was not large enough to represent all AAV patients in Korea or to strengthen the reliability of our results. Due to the cross-sectional study design, it was impossible to analyze the dynamic correlation between serum FSTL1 and SF-36 at different time points. The measurement of pro-inflammatory cytokines could have supported our hypothesis pertaining to the correlation of serum FSTL1 with BVAS and SF-36, but it was not measured in this study. Also, we could not include positive controls in this study owing to a small number of age- and gender-matched positive controls. Moreover, it might be a correlation coefficient that can be judged fair if it ranges from 0.3 to 0.5,^[23] however, the correlation coefficients of FSTL1 with SF-36 PCS and SF-36 MCS were not high enough to support its potential as a biomarker for estimating the functional status of AAV. Therefore, although SF-36 PCS and MCS are indicators related to quality-of-life, which are mixtures of subjective and objective contents, a higher correlation coefficient should be validated by a larger number of AAV patients. We believe that the findings of this pilot study revealing the clinical implications of serum FSTL1 in AAV for the first time will have an impact on clinical practice. Furthermore, we expect that future prospective studies including a larger population, analyses of paired blood samples, and clinical data will provide valuable information on the dynamic clinical significance as well as the potential of serum FSTL1 for predicting prognosis in AAV patients.

We conclude that serum FSTL1 could predict the current both SF-36 PCS and MCS in AAV patients. We suggest that serum FSTL1 may be used for estimating the current functional status of AAV in patients who have difficulties in completing the SF-36 forms, not only for assessing HRQOL but also for evaluating the achievement of the therapeutic goals.

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Conflicts of interest

None.

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