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Vasculitis Activity-Predicting Ability of IL-12 Family Cytokines in Patients with Microscopic Polyangiitis and Granulomatosis with Polyangiitis

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Purpose: The present study investigated and compared the antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) activity-predicting ability of the serum concentrations of the four interleukin (IL)-12 family cytokines including IL-23, IL-27, IL-35, and IL-39 in patients with microscopic polyangiitis (MPA) and granulomatosis with polyangiitis (GPA).

Materials and Methods: The present study included 70 patients with MPA and GPA. Clinical and laboratory data, particularly Birmingham Vasculitis Activity Score (BVAS), at the time of blood collection were obtained. The serum concentrations of IL-23, IL-27, IL-35, and IL-37 were measured using sera stored at -80°C. Patients were divided into two groups: the upper half of BVAS (BVAS ≥12) and the lower half of BVAS (BVAS <12).

Results: The serum concentrations of IL-23 and IL-27 reflected AAV activity. Patients with the upper half of BVAS exhibited significantly higher serum concentrations of IL-23 and IL-27 than those without. Patients with the serum concentrations of IL-23 ≥132.1 pg/mL or IL-27 ≥684.7 pg/mL exhibited higher frequency and risk for the upper half of BVAS than those without [relative risks (RR) 5.143 and RR 4.091, respectively]. The serum concentrations of IL-27 were associated with age ≥65 years and proteinase 3-ANCA (or C-ANCA) negativity, whereas, those of IL-23 were associated with MPA. However, the serum concentrations of IL-35 and IL-39 were not useful in predicting AAV activity in this study.

Conclusion: The present study is the first to demonstrate that among the various members of IL-12 family cytokines, the serum concentrations of IL-23 and IL-27 possess AAV activity-predicting ability.

Key Words: Microscopic polyangiitis, granulomatosis with polyangiitis, IL-12 family cytokines, activity, predict

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INTRODUCTION

The interleukin (IL)-12 family cytokines including IL-12, IL-23, IL-27, IL-35, and IL-39, are heterodimeric, each having two unique subunits and distinct transmembrane receptors.[1,2] IL-12 (p35 and p40) enhances interferon-γ production and TH1 cell differentiation via the signal transducer and activator of transcription (STAT)4/STAT4 homodimer, whereas, IL-23 (p19 and p40) promotes IL-17 production and TH17 cell differentiation via the STAT3/STAT4 heterodimer. On the other hand, IL-27 [p28 and Epstein-Barr virus induced 3 (EBI3)] has dual actions via the STAT1/STAT3 heterodimer: it accelerates overall T cell responses but inhibits TH17 cell function by augmenting IL-10 production of Treg cells.[1,3] Conversely, IL-35

(p35 and EBI3), secreted by Treg, Breg, and CD8+ Treg cells, induces Treg cell proliferation and decreases TH17 cell differentiation via the STAT1/STAT4 heterodimer. In addition, IL-35 enhances the production of IL-35-producing induced regulatory T cells (iTr35).[1,3,4] IL-39 (p19 and EBI3), a novel IL-12 family cytokine, activates B cells and increases the expression of adhesion molecules such as vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 on the endothelial cell surface via the STAT1/STAT3 heterodimer.[5] Previous studies have indicated that the serum concentration of each IL-12 family cytokine has clinical implications in reflecting the activity of autoimmune diseases.[6-9]

Antineutrophil cytoplasmic antibodies (ANCAs) circulating in the bloodstream can lead to ANCA-associated vasculitis (AAV), an autoimmune disease that causes inflammation and narrowing of arterioles, venules, capillaries, and even medium-sized arteries.[10,11] The priming and activation of neutrophils by autoreactive TH1 and TH17 cells and the production of circulating ANCAs by B cells are critical for the pathogenesis of AAV.[12,13] In this context, it could be reasonably assumed that the serum concentrations of IL-12 family cytokines modulate AAV activity. Previous studies have shown a significant correlation between IL-23/IL-27 serum concentrations and the degree of AAV activity.[14,15] However, those studies only probed the AAV activity-predicting ability of a particular cytokine while ignoring intra-family comparisons between various members of IL-12 family cytokines in this respect. In addition, those studies did not perform any subgroup analyses. Hence, the present study investigated and compared the AAV activity-predicting ability of the serum concentrations of four IL-12 family cytokines, including IL-23, IL-27, IL-35, and IL-39, in patients with microscopic polyangiitis (MPA) and granulomatosis with polyangiitis (GPA). Furthermore, we performed subgroup analyses with respect to sex, age ≥65 years, AAV subtype, and ANCA type.

MATERIALS AND METHODS

Study subjects

The present study included 70 patients who were first diagnosed with MPA and GPA at this university-affiliated tertiary hospital. All patients met the 2007 European Medicine Agency algorithm for AAV, and the revised 2012 Chapel Hill Consensus Conference for vasculitis nomenclature.[10,11] They also fulfilled the 2022 American College of Rheumatology and the European Alliance of Associations for Rheumatology classification criteria for AAV (the 2022 ACR/EULAR criteria).[16-18] The participants' medical records were detailed, providing clinical, laboratory, radiologic, and histological data on suspicion and confirmation of AAV diagnosis; and data at the time of blood collection. In addition, patients who had concurrent malignancies, serious infectious diseases, or other diseases mimicking AAV at the time of initiation of this study were excluded.[16,17]

Ethical disclosure

This study was approved by the Institutional Review Board (IRB) of Severance Hospital, Seoul, Republic of Korea (Approval number 4-2022-1439), and written informed consent was obtained from all patients at the time of blood collection. The IRB waived the need for written informed consent from patients who had provided it during entry into the cohort.

Determination of serum ANCA titres

According to the 2022 ACR/EULAR criteria for MPA and GPA, myeloperoxidase (MPO)-ANCA and proteinase 3 (PR3)-ANCA measured by an immunoassay and perinuclear (P)-ANCA and cytoplasmic (C)-ANCA detected by an indirect immunofluorescence assay were accepted for quantifying circulating ANCA titres.[16,17] When patients had no MPO-ANCA or PR3-ANCA but had P-ANCA or C-ANCA, and when AAV was strongly suspected, they were considered as having ANCA.[19,20]

AAV-specific indices

Birmingham Vasculitis Activity Score (BVAS), Five-Factor Score (FFS), the Korean version of the Short-Form 36-Item Health Survey (SF-36) physical component summary (PCS), and mental component summary (MCS), and Vasculitis Damage Index (VDI) were assessed as AAV-specific indices.[21-24]

Upper and lower half of BVAS

By using the median of BVAS, all patients were divided into two groups: those with the upper half of BVAS (BVAS ≥12) and the lower half of BVAS (BVAS <12).

Clinical and laboratory data

At the time of blood collection, age and sex were collected as demographic data. The presence of systemic manifestation and their calculated total scores were based on BVAS.[17] Laboratory tests included quantification of white blood cell and platelet counts, haemoglobin, total serum protein, serum albumin, blood urea nitrogen, serum creatinine levels, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels.

Quantification of the serum concentrations of IL-12 family cytokines

The serum concentrations of IL-23 and IL-27 were measured using the Human Magnetic Luminex assay (R&D Systems, Minneapolis, MN, USA). The serum concentrations of IL-35 and IL-39 were measured using the enzyme-linked immunosorbent assay kits (MyBioSource, San Diego, CA, USA). Both quantifications were performed according to the instructions of the manufacturers and by using sera samples stored at -80°C.

Statistical analyses

All statistical analyses were performed using the SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, NY, USA). Continuous and categorical variables are expressed as medians with

25–75 percentiles and numbers (percentages), respectively. Correlation coefficients (r) between two variables were obtained using Pearson correlation analysis. Optimal cut-off values were extrapolated by performing receiver operating characteristic (ROC) curve analysis, and values with the maximised sum of sensitivity and specificity were selected. The relative risks (RR) of the cut-off values for the upper half of BVAS were analysed using contingency tables and the chi-square test. Significant differences between two continuous and categorical variables were compared using the Mann-Whitney U test, and the chi-square and Fisher's exact tests, respectively. Odds ratio (OR) were obtained using multivariable logistic regression analysis with variables with $p<0.005$ in univariable logistic regression analysis. p-values<0.05 were considered statistically significant.

RESULTS

Characteristics of patients at the time of blood collection

The median age of the patients was 67.0 years (male 40.0%). Of the 70 patients, 42 and 28 patients were diagnosed with MPA and GPA, respectively. MPO-ANCA (or P-ANCA) and PR3-ANCA (or C-ANCA) were positive in 53 and 11 patients, respectively. The median BVAS, FFS, SF-36 PCS and MCS, and VDI values were 12.0, 2.0, 45.9, 51.8, and 3.0, respectively. The most common systemic manifestation was pulmonary manifestation (68.6%), followed by renal manifestation (60.0%). The median serum concentrations of IL-23, IL-27, IL-35, and IL-39, were 182.5, 655.0, 52.8, and 48.2 pg/mL, respectively (Table 1).

Correlations between the serum concentrations of IL-12 family cytokines and AAV-specific indices or acute-phase reactants

Among four IL-12 family cytokines, the serum concentration of IL-23 was significantly correlated with BVAS (r=0.286), SF-36 PCS (r=-0.253), SF-36 MCS (r=-0.245), ESR (r=0.465), and CRP (r=0.492). The serum concentration of IL-27 was significantly correlated with BVAS (r=0.336), FFS (r=0.337), SF-36 PCS (r=-0.286), SF-36 MCS (r=-0.253), and CRP (r=0.452). The serum concentrations of IL-35 or IL-39 were not correlated with AAV-specific indices or acute-phase reactants (Table 2).

Comparison of the serum concentrations of four IL-12 family cytokines between AAV patients with the upper half of BVAS and those with the lower half of BVAS

Among four IL-12 family cytokines, patients with the upper half of BVAS exhibited significantly higher median serum con-

Table 1. Characteristics of Patients at the Time of Blood Collection (n=70)

Variables	Values
Demographic data	
Age (yr)	67.0 (55.8–73.0)
Male sex	28 (40.0)
AAV subtypes	
MPA	42 (60.0)
GPA	28 (40.0)
ANCA positivity	
MPO-ANCA (or P-ANCA) positive	53 (75.7)
PR3-ANCA (or C-ANCA) positive	11 (15.7)
AAV-specific indices	
BVAS	12.0 (6.8–18.3)
FFS	2.0 (1.0–2.0)
SF-36 PCS	45.9 (25.9–62.7)
SF-36 MCS	51.8 (34.4–66.4)
VDI	3.0 (2.0–4.0)
Systemic items of BVAS	
General manifestation	26 (37.1)
Cutaneous manifestation	10 (14.3)
Mucous and ocular manifestation	3 (4.3)
Otorhinolaryngologic manifestation	26 (37.1)
Pulmonary manifestation	48 (68.6)
Cardiovascular manifestation	5 (7.1)
Gastrointestinal manifestation	1 (1.4)
Renal manifestation	42 (60.0)
Nervous systemic manifestation	17 (24.3)
Serum concentrations of IL-12 family cytokines (pg/mL)	
IL-23	182.5 (56.9–306.1)
IL-27	655.0 (411.2–948.0)
IL-35	52.8 (35.8–144.8)
IL-39	48.2 (34.1–115.4)

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, Birmingham Vasculitis Activity Score; FFS, Five-Factor Score; SF-36, Short-Form 36-Item Health Survey; PCS, physical component summary; MCS, mental component summary; VDI, Vasculitis Damage Index; IL, interleukin.
Values are expressed as a median (25–75 percentile) or n (%).

Table 2. Correlation of the Serum Concentrations of Four IL-12 Family Cytokines with AAV-Specific Indices and Acute-Phase Reactants

	BVAS	FFS	SF-36 PCS	SF-36 MCS	VDI	ESR	CRP
IL-23	0.286 (0.017)	0.126 (0.297)	-0.253 (0.036)	-0.245 (0.042)	0.057 (0.642)	0.465 (<0.001)	0.492 (<0.001)
IL-27	0.336 (0.004)	0.337 (0.004)	-0.286 (0.017)	-0.253 (0.036)	0.191 (0.113)	0.195 (0.106)	0.452 (<0.001)
IL-35	0.076 (0.530)	0.128 (0.289)	0.090 (0.463)	0.031 (0.798)	0.068 (0.576)	-0.007 (0.925)	-0.015 (0.901)
IL-39	-0.214 (0.075)	0.047 (0.699)	-0.022 (0.856)	-0.031 (0.801)	-0.075 (0.537)	-0.115 (0.343)	0.019 (0.878)

IL, interleukin; ANCA, antineutrophil cytoplasmic antibody; AAV, ANCA-associated vasculitis; BVAS, Birmingham Vasculitis Activity Score; FFS, Five-Factor Score; SF-36, Short-Form 36-Item Health Survey; PCS, physical component summary; MCS, mental component summary; VDI, Vasculitis Damage Index; ESR, rythrocyte sedimentation rate; CRP, C-reactive protein.
Values are expressed as a correlation coefficient (p-value).

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centrations of IL-23 (240.0 pg/mL vs. 100.8 pg/mL) and IL-27 (802.7 pg/mL vs. 488.5 pg/mL) than those with the lower half of BVAS. However, there were no significant differences in the serum concentrations of IL-35 and IL-39 between the two groups (Fig. 1).

Optimal cut-off values for the serum concentrations of IL-23 and IL-27 for the upper half of BVAS

When ROC curve analysis was performed, the area under the curve (AUC) values of the serum concentrations of IL-23 and IL-27 was statistically significant. When the cut-off of the serum concentration of IL-23 for the upper half of BVAS was set as 132.1 pg/mL, the sensitivity and specificity were 75.0% and 63.2%, respectively. In addition, when that of IL-27 for the upper half of BVAS was set as 684.7 pg/mL, the sensitivity and specificity were 62.5% and 71.1%, respectively (Fig. 2A and C).

RRs for the upper half of BVAS

When we divided patients into two groups based on the serum concentration of IL-23 ≥132.1 pg/mL, the upper half of BVAS was identified more frequently in patients with the serum concentrations of IL-23 ≥132.1 pg/mL than those without (63.2% vs. 25.0%, p=0.001). Furthermore, patients with the serum concentrations of IL-23 ≥132.1 pg/mL exhibited a significantly higher risk for the upper half of BVAS than those without [RR 5.143, 95% confidence interval (CI) 1.824–14.502] (Fig. 2B). Similarly, patients with the serum concentrations of IL-27 ≥684.7 pg/mL showed a higher frequency and risk for the upper half of BVAS than those without (64.5% vs. 30.8%, p=0.005; RR 4.091, 95% CI 1.502–11.141) (Fig. 2D).

Subgroup analyses according to sex, age ≥65 years, AAV subtype, and ANCA type

With respect to the sex of the patients, there were no significant differences in the serum concentrations of IL-12 family cytokines between male and female patients. Meanwhile, patients of age ≥65 years showed a significantly higher median serum concentration of IL-27 than those of age <65 years (832.0 pg/mL vs. 473.2 pg/mL, p=0.001). Given the AAV subtype, MPA patients exhibited a significantly higher median serum concentration of IL-23 than GPA patients (221.4 pg/mL vs. 100.8 pg/mL, p=0.038). In the context of ANCA type, no significant differences in the serum concentration of IL-12 family cytokines were noted between patients with MPO-ANCA (or P-ANCA) and those without; however, patients with PR3-ANCA (or C-ANCA) had a significantly lower median serum concentration of IL-27 than those without PR3-ANCA (or C-ANCA) (348.3 vs. 655.0, p=0.013) (Fig. 3).

DISCUSSION

In this study, we investigated and compared the AAV activity-predicting ability of the serum concentrations of four IL-12 family cytokines, namely IL-23, IL-27, IL-35, and IL-39, in MPA and GPA patients. We also performed subgroup analyses with

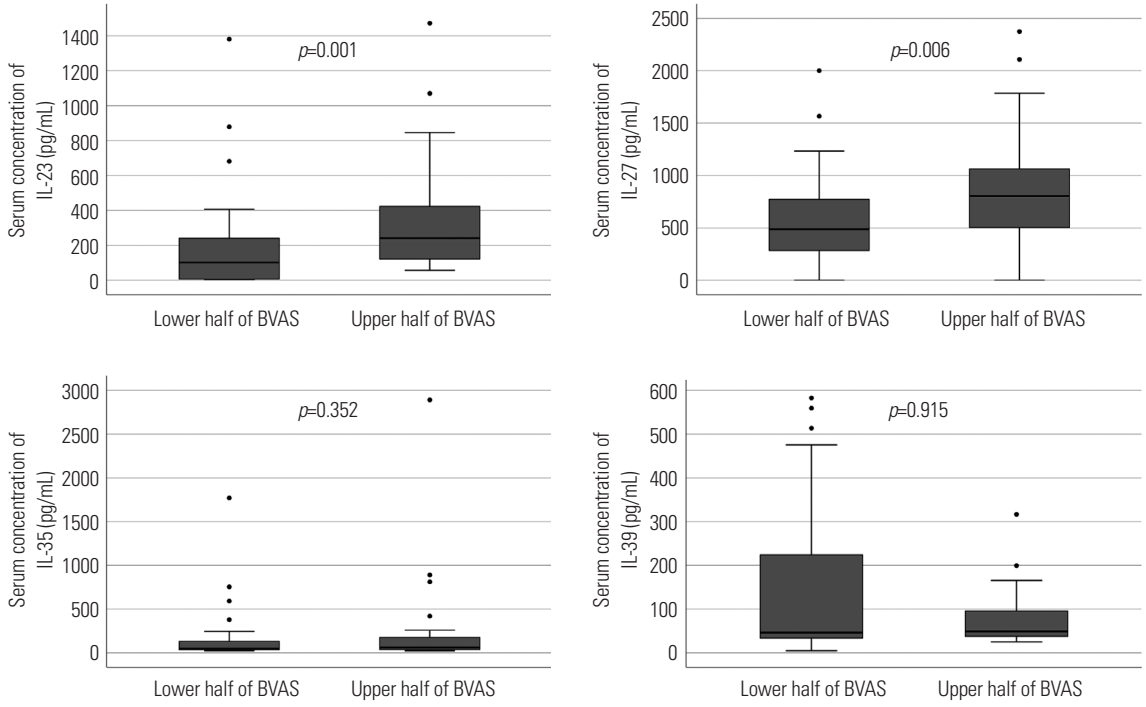

Fig. 1. Comparison of the four serum concentrations of IL-12 family cytokines between AAV patients with the upper half of BVAS and those with the lower half of BVAS. IL, interleukin; AAV, antineutrophil cytoplasmic antibody-associated vasculitis; BVAS, Birmingham Vasculitis Activity Score.

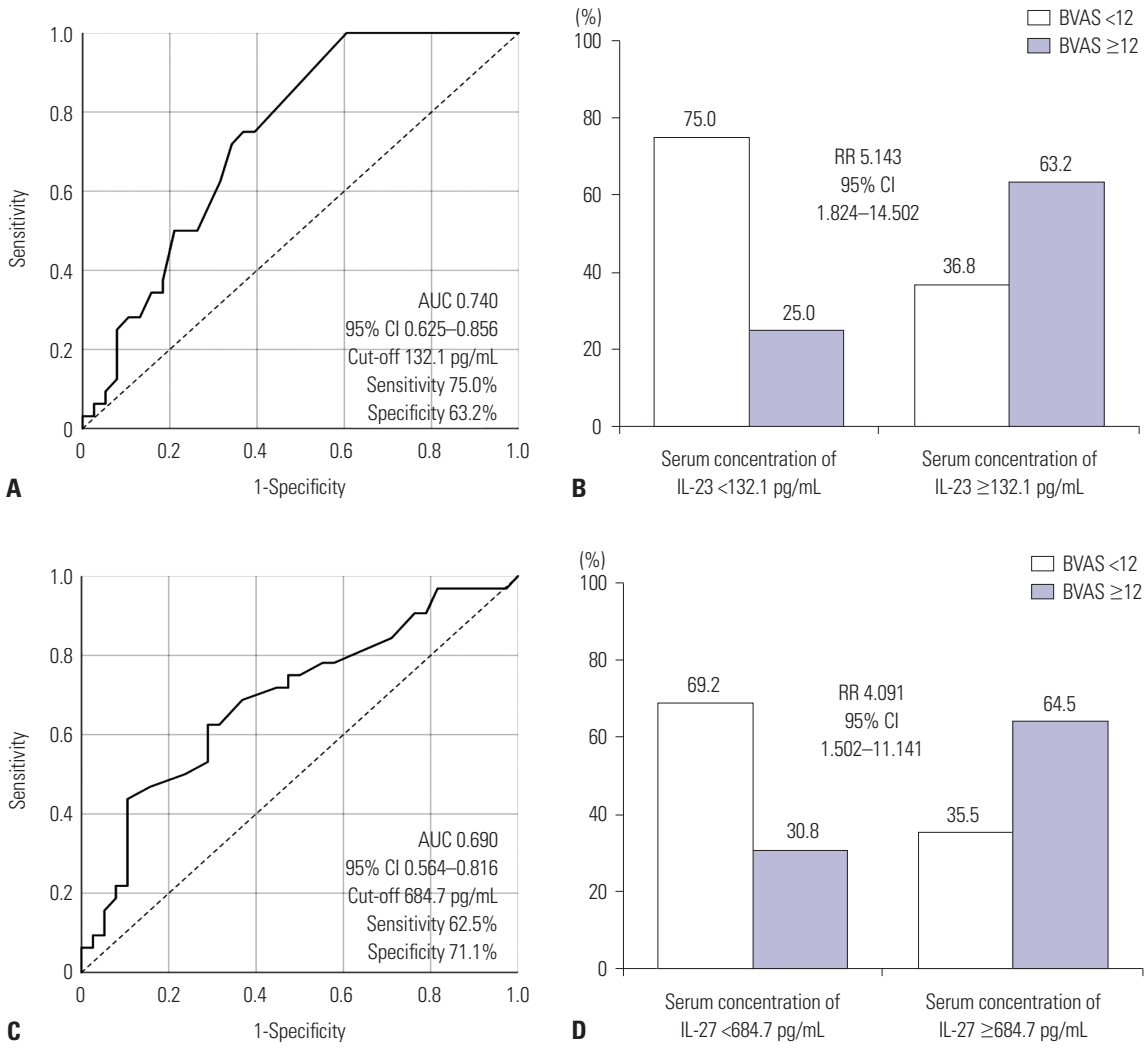

Fig. 2. Cut-off values of the serum concentrations of IL-23 and IL-27 for the upper half of BVAS and their RR. (A) ROC curve of the serum concentration of IL-23, (B) RR of the serum concentration of IL-23 over the cut-off for the upper half of BVAS, (C) ROC curve of the serum concentration of IL-27, and (D) RR of the serum concentration of IL-27 over the cut-off for the upper half of BVAS. IL, interleukin; BVAS: Birmingham Vasculitis Activity Score; RR, relative risk; AUC, area under the curve; CI, confidence interval.

reference to sex, age ≥65 years, AAV subtype, and ANCA type. First, among the four serum concentrations of IL-12 family cytokines, the serum concentrations of IL-23 and IL-27 reflected the degree of activity of MPA and GPA in addition to ESR and CRP. Second, when divided into two BVAS-based groups, patients with the upper half of BVAS exhibited significantly higher median serum concentrations of IL-23 and IL-27 than those with the lower half of BVAS but not those of IL-35 and IL-39. Third, when the optimal cut-offs of the serum concentrations of IL-23 and IL-27 for the upper half of BVAS were determined using the ROC curve, patients with the serum concentrations of IL-23 ≥132.1 pg/mL and those with the serum concentrations of IL-27 ≥684.7 pg/mL exhibited higher frequency and risk for the upper half of BVAS than those without (RR 5.143, p=0.001 and RR 4.091, p=0.005, respectively). Fourth, in subgroup analyses, the serum concentrations of IL-27 were significantly associated with age ≥65 years and PR3-ANCA (or C-ANCA) negativity. Meanwhile, the serum concentrations of IL-23 were significantly associated with MPA but not MPO-ANCA (or P-ANCA). Therefore, we concluded that both the serum concentrations of IL-23 and IL-27 had AAV-activity predicting ability in MPA and GPA patients and are more likely to be associated with MPA and age ≥65 years, respectively.

Among four IL-12 family cytokines, both IL-23 and IL-27 are primarily produced and secreted by activated macrophages and dendritic cells, which play pivotal roles in the pathogenesis of AAV.[14,15,25,26] Secreted IL-23 has the unidirectional immune property of inducing TH17 cell polarisation and maintaining its pathogenicity.[27] Meanwhile, secreted IL-27 has the bidirectional immune property of participating in pro-inflammatory and anti-inflammatory processes.[28] The results of this study suggest that the pro-inflammatory function of secreted IL-27 is superior to its anti-inflammatory function. Additionally, IL-27 was reported to act as a turn-on signal for IL-23.[29] Next, since IL-35 is

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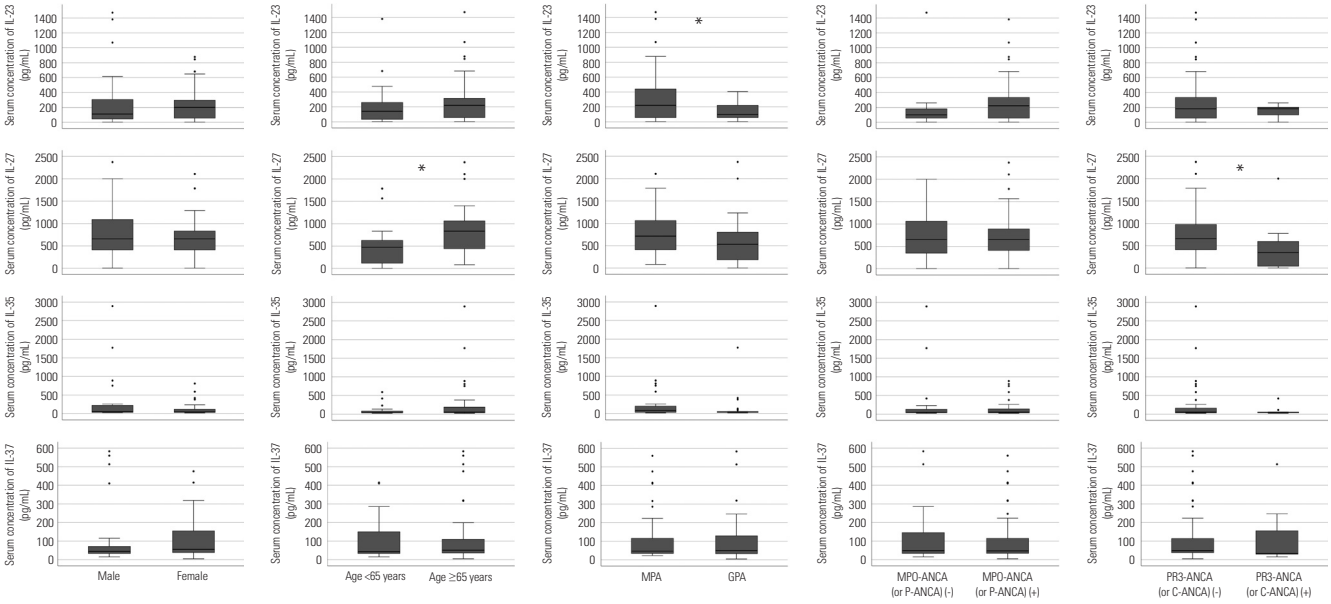

Fig. 3. Comparison of the four serum concentrations of IL-12 family cytokines according to sex, age ≥65 years, AAV subtype and ANCA type. *statistically significant. IL, interleukin; AAV, ANCA-associated vasculitis; ANCA, antineutrophil cytoplasmic antibody; MPA, microscopic polyangiitis; GPA, granulomatosis with polyangiitis; MPO, myeloperoxidase; P, perinuclear; PR3, proteinase 3; C, cytoplasmic.

mainly produced and secreted by Treg and Breg cells, secreted IL-35 has been reported to play immune-suppression roles in various autoimmune diseases.[30] Recently, IL-39 has been introduced as a novel pro-inflammatory cytokine.[27] Therefore, the serum concentrations of IL-35 and IL-39 were expected to be inversely and positively correlated with BVAS, respectively but they had no significant correlation with BVAS in MPA and GPA patients. Taken together with the results of this study, we concluded that, among four IL-12 family cytokines, the serum concentrations of IL-23 and IL-27 have predictive potential for high AAV activity in MPA and GPA patients.

Additionally, the serum concentrations of IL-23 and IL-27 exhibited divergent associations with age ≥65 years, AAV subtypes, and ANCA type (Fig. 2). Regarding age ≥65 years, the serum concentration of IL-27 was reported to increase with age in patients with rheumatoid arthritis (RA) but not in healthy controls.[31] Although RA is not the same disease as AAV, the above report may support our findings because these two medical conditions are autoimmune inflammatory diseases. Regarding the AAV subtype and ANCA type, it was novel and interesting that the serum concentration of IL-23 was higher in MPA patients, and that of IL-27 was higher in PR3-ANCA-negative patients. This result may be valid because the patients included in this study also met the 2022 ACR/EULAR criteria for MPA and GPA.[16,17] Based on the new criteria, it is very difficult for PR3-ANCA-positive patients to be diagnosed with MPA. Therefore, although no differences were observed according to the presence or absence of MPO-ANCA, it may be inferred that both serum concentrations of IL-23 and IL-27 may be more closely related to the pathogenesis of MPA than that of GPA.

First, the ROC curve analysis was performed for comparing AUCs for the upper half of BVAS between the serum concentrations of IL-23 and IL-27: the AUC of the serum concentration of IL-23 was slightly higher than that of IL-27 in MPA and GPA patients (AUC 0.740, 95% CI 0.625–0.856 for IL-23 vs. AUC 0.690, 95% CI 0.564–0.816 for IL-27) (Fig. 2). Logistic regression analysis was performed to compare the predicting ability for the upper half of BVAS between the serum concentrations of IL-23 ≥132.1 pg/mL and the serum concentrations of IL-27 ≥684.7 pg/mL. In univariable analysis, both serum concentrations of IL-23 ≥132.1 pg/mL (OR 5.143, 95% CI 1.824–14.502, p=0.002) and IL-27 ≥684.7 pg/mL (OR 4.091, 95% CI 1.502–11.141, p=0.006) were significantly associated with the upper half of BVAS. In multivariable analysis, both of them also were significantly associated with the upper half of BVAS (OR 4.699, 95% CI 1.589–13.894 and OR 3.684, 95% CI 1.265–10.734, respectively) (Supplementary Table 1, only online). Taken together, we concluded that both serum concentrations of IL-23 ≥132.1 pg/mL and IL-27 ≥684.7 pg/mL were independently associated with the upper half of BVAS and that the serum concentrations of IL-23 tended to exhibit a slightly better AAV activity-predicting ability than those of IL-27, despite no statistical significance.

Although we previously discovered an association between the serum concentrations of IL-27 and AAV activity, we did not compare AAV activity-predicting ability among four IL-12 family cytokines.[15] Therefore, it could be considered that the present study is the first to demonstrate that both serum concentrations of IL-23 and IL-27 reflect the cross-sectional AAV activity in MPA and GPA patients. The present study has clinical implications in that it provides a method to obtain the cut-offs of the serum concentrations of IL-23 and IL-27 and RR values for individuals with the upper half of BVAS. Also, our findings have

the potential to apply these findings to populations with ethnic and regional differences.

The present study has several limitations. First, the relatively small number of patients could not enable the results of this study to be applied to MPA and GPA patients immediately: in particular, the small sample size might reduce the reliability of the results due to the relatively low statistical power of the subgroup analyses. Although well-documented medical records and stored blood samples were available, this was a retrospective study that could not exclude missing or inaccurate data. Moreover, the sensitivity and specificity of the serum concentrations of IL-23 and IL-27 for the upper half of BVAS of MPA and GPA were not as high as to be independently applied to real clinical settings. However, we believe that the present study suggests two potentially significant research opportunities: one would be to discover the mechanisms of how IL-23 and IL-27 participate in the pathogenesis of MPA and GPA, and the other would be to add new biomarkers to objectively estimate AAV activity regardless of the time of measurement or the measuring physicians. In addition, the present study has one critical limitation: since we did not measure the serum concentrations of IL-12 family cytokines serially as the cross-sectional activity of MPA and GPA changed, we could not determine the dynamic concordance or discordance of both BVAS and the serum concentrations of IL-12 family cytokines. This may weaken the conclusion of the present study; however, we believe that our results suggest their potential usefulness in real clinical settings as an initial pilot study. We also believe that a prospective future study that includes more MPA and GPA patients and measures the serum concentrations of IL-12 family cytokines serially according to BVAS assessment at different time points will provide more reliable and reproducible data regarding their activity-predicting abilities.

In conclusion, the present study is the first to demonstrate that among the various members of IL-12 family cytokines, the serum concentrations of IL-23 and IL-27 possess AAV-activity predicting ability and support the association of the serum concentrations of IL-27 in older adults and the serum concentrations of IL-23 with MPA patients. Therefore, data from the present study suggest that measuring the serum concentrations of IL-23 and IL-27 could help to assess the degree of AAV activity and follow up on it.

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AUTHOR CONTRIBUTIONS

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

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