Title	Seasonal Influence on Development of Antineutrophil Cytoplasmic Antibody-Associated Vasculitis: A Retrospective Cohort Study Conducted at Multiple Institutions in Japan (J-CANVAS)
Author(s)	Yoshida, Yusuke; Nakamoto, Naoki; Oka, Naoya; Kidoguchi, Genki; Hosokawa, Yohei; Araki, Kei; Ishitoku, Michinori; Watanabe, Hirofumi; Sugimoto, Tomohiro; Mokuda, Sho; Kida, Takashi; Yajima, Nobuyuki; Omura, Satoshi; Nakagomi, Daiki; Abe, Yoshiyuki; Kadoya, Masatoshi; Takizawa, Naoho; Nomura, Atsushi; Kukida, Yuji; Kondo, Naoya; Yamano, Yasuhiko; Yanagida, Takuya; Endo, Koji; Matsui, Kiyoshi; Takeuchi, Tohru; Ichinose, Kunihiro; Kato, Masaru; Yanai, Ryo; Matsuo, Yusuke; Shimojima, Yasuhiro; Nishioka, Ryo; Okazaki, Ryota; Takata, Tomoaki; Ito, Takafumi; Moriyama, Mayuko; Takatani, Ayuko; Miyawaki, Yoshia; Ito-Ihara, Toshiko; Kawaguchi, Takashi; Kawahito, Yutaka; Hirata, Shintaro
Citation	The Journal of Rheumatology , 50 (9) : 1152 - 1158
Issue Date	2023-09-01
DOI	
Self DOI	
URL	https://ir.lib.hiroshima-u.ac.jp/00056137
Right	Copyright © 2023 by the Journal of Rheumatology This is a pre-copyediting, author-produced PDF of an article accepted for publication in The Journal of Rheumatology following peer review. The definitive publisher-authenticated version Seasonal Influence on Development of Antineutrophil Cytoplasmic Antibody-Associated Vasculitis: A Retrospective Cohort Study Conducted at Multiple Institutions in Japan (J-CANVAS),

The Journal of Rheumatology

Seasonal influence on development of anti-neutrophil cytoplasmic antibody-associated vasculitis: a retrospective cohort study conducted at multiple institutions in Japan (J-CANVAS)

Journal:	*The Journal of Rheumatology*
Manuscript ID	2023-0040.R2
Manuscript Type:	Manuscript
Date Submitted by the Author:	n/a
Complete List of Authors:	Yoshida, Yusuke; Hiroshima University Hospital, Department of Clinical Immunology and Rheumatology Nakamoto, Naoki; Hiroshima University Hospital, Department of Clinical Immunology and Rheumatology Oka, Naoya; Hiroshima University Hospital, Department of Clinical Immunology and Rheumatology Kidoguchi, Genki; Hiroshima University Hospital, Department of Clinical Immunology and Rheumatology Hosokawa, Yohei; Hiroshima University Hospital, Department of Clinical Immunology and Rheumatology Araki, Kei; Hiroshima University Hospital, Department of Clinical Immunology and Rheumatology Ishitoku, Michinori; Hiroshima University Hospital, Department of Clinical Immunology and Rheumatology Watanabe, Hirofumi; Hiroshima University Hospital, Department of Clinical Immunology and Rheumatology Sugimoto, Tomohiro; Hiroshima University Hospital, Department of Clinical Immunology and Rheumatology Mokuda, Sho; Hiroshima University Hospital, Department of Clinical Immunology and Rheumatology Kida, Takashi; Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Inflammation and Immunology Yajima, Nobuyuki; Showa University School of Medicine, Division of Rheumatology, Department of Medicine Omura, Satoshi; Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Inflammation and Immunology Nakagomi, Daiki; University of Yamanashi Hospital, Department of Rheumatology Abe, Yoshiyuki; Juntendo University, Department of Internal Medicine and Rheumatology Kadoya, Masatoshi; Japanese Red Cross Society Kyoto Daiichi Hospital, Center for Rheumatic Disease Takizawa, Naoho; Chubu Rosai Hospital, Department of Rheumatology Nomura, Atsushi; St. Luke's International Hospital, Immuno-Rheumatology Center Kukida, Yuji; Japanese Red Cross Society Kyoto Daini Hospital, Department of Rheumatology

	Kondo, Naoya; Kyoto Katsura Hospital, Department of Nephrology Yamano, Yasuhiko; Tosei General Hospital, Department of Respiratory Medicine and Allergy Yanagida, Takuya; Kagoshima University Hospital, Department of Hematology and Rheumatology Endo, Koji; Tottori Prefectural Central Hospital, Department of General internal medicine; Kyoto University, Department of Pharmacoepidemiology, graduate School of Medicine and Public Health Matsui, Kiyoshi; Hyogo Medical University, School of Medicine, Department of Diabetes, Endocrinology and Clinical Immunology Takeuchi, Tohru; Osaka Medical and Pharmaceutical University, Department of Internal Medicine (IV) Ichinose, Kunihiro; Nagasaki University Graduate School of Biomedical Sciences, Department of Immunology and Rheumatology, Division of Advanced Preventive Medical Sciences; Shimane University Faculty of Medicine, Department of Rheumatology Kato, Masaru; Graduate School of Medicine, Hokkaido University, Department of Rheumatology, Endocrinology and Nephrology Yanai, Ryo; Showa University School of Medicine, Division of Rheumatology, Department of Medicine Matsuo, Yusuke; Tokyo Kyosai Hospital, Department of Rheumatology; Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), Department of Rheumatology Shimojima, Yasuhiro; Shinshu University School of Medicine, Department of Medicine (Neurology and Rheumatology) Nishioka, Ryo; Graduate School of Medical Science, Kanazawa University, Department of Rheumatology Okazaki, Ryota; Tottori University, Division of Respiratory Medicine and Rheumatology, Department of Multidisciplinary Internal Medicine, Faculty of Medicine Takata, Tomoaki; Tottori University, Division of Gastroenterology and Nephrology Ito, Takafumi; Shimane University Hospital, Division of Nephrology Moriyama, Mayuko; Shimane University Faculty of Medicine, Department of Rheumatology Takatani, Ayuko; Sasebo Chuo Hospital, Rheumatic Disease Center Miyawaki, Yoshia; Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Department of Nephrology, Rheumatology, Endocrinology and Metabolism Ito-Ihara, Toshiko; University Hospital, Kyoto Prefectural University of Medicine, The Clinical and Translational Research Center Kawaguchi, Takashi; Tokyo University of Pharmacy and Life Sciences, Department of Practical Pharmacy Kawahito, Yutaka; Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Inflammation and Immunology Hirata, Shintaro; Hiroshima University Hospital, Deparment of Clinical Immunology and Rheumatology

Keywords:	Antineutrophil cytoplasmic antibodies, Environment, Eosinophilic granulomatosis with polyangiitis, Granulomatosis with Polyangiitis

Short running head: Seasonality of ANCA

Full title of manuscript: Seasonal influence on development of anti-neutrophil cytoplasmic antibody-associated vasculitis: a retrospective cohort study conducted at multiple institutions in Japan (J-CANVAS)

Authors: Yusuke Yoshida[1*], Naoki Nakamoto[1], Naoya Oka[1], Genki Kidoguchi[1], Yohei Hosokawa[1], Kei Araki[1], Michinori Ishitoku[1], Hirofumi Watanabe[1], Tomohiro Sugimoto[1], Sho Mokuda[1], Takashi Kida[2], Nobuyuki Yajima[3], Satoshi Omura[2], Daiki Nakagomi[4], Yoshiyuki Abe[5], Masatoshi Kadoya[6], Naoho Takizawa[7], Atsushi Nomura[8], Yuji Kukida[9], Naoya Kondo[10], Yasuhiko Yamano[11], Takuya Yanagida[12], Koji Endo[13,14], Kiyoshi Matsui[15], Tohru Takeuchi[16], Kunihiro Ichinose[17,18], Masaru Kato[19], Ryo Yanai[3], Yusuke Matsuo[20,21], Yasuhiro Shimojima[22], Ryo Nishioka[23], Ryota Okazaki[24], Tomoaki Takata[25], Takafumi Ito[26], Mayuko Moriyama[18], Ayuko Takatani[27], Yoshia Miyawaki[28], Toshiko Ito-Ihara[29], Takashi Kawaguchi[30], Yutaka Kawahito[2], and Shintaro Hirata[1]

ORCID iD

Y. Yoshida: 0000-0002-0774-0509

T. Sugimoto: 0000-0002-0968-9081

S. Mokuda: 0000-0002-8529-1016

T. Kida: 0000-0002-5740-8352

N. Yajima: 0000-0002-8292-3962

D. Nakagomi: 0000-0003-1682-9799

Y. Abe: 0000-0002-1014-2868

N. Takizawa: 0000-0003-3744-3537

A. Nomura: 0000-0002-8194-6273

Y. Kukida: 0000-0003-4074-4533

K. Matsui: 0000-0002-1146-4172

K. Ichinose: 0000-0001-8794-7106

Y Shimojima: 0000-0001-7100-1121

T. Takata: 0000-0003-2959-6015

T. Ito: 0000-0003-0677-6566

Y. Miyawaki: 0000-0002-6981-3443

T. Ito-Ihara: 0000-0001-7152-3026

T. Kawaguchi: 0000-0003-2446-7716

Y. Kawahito: 0000-0002-3860-0488

S. Hirata: 0000-0002-2474-9943

Sources of support in the form of grants or industrial support: This work was supported in part by JSPS KAKENHI (Grant Numbers 19K18499 and 22K08599 to S.M.).

Initials, surnames, appointments, and highest academic degrees of all authors

Y. Yoshida[1], MD, PhD; N. Nakamoto[1], MD; N. Oka[1], MD; G. Kidoguchi[1], MD; Y. Hosokawa[1], MD; K Araki[1], MD; M Ishitoku[1], MD; H Watanabe[1], MD; T. Sugimoto[1], MD, PhD; S. Mokuda[1], MD, PhD; T. Kida[2], MD, PhD, MPH; N. Yajima[3], MD, PhD, MPH; S. Omura[2], MD; D. Nakagomi[4], MD, PhD; Y. Abe[5], MD, PhD; M. Kadoya[6], MD, PhD; N. Takizawa[7], MD; A. Nomura[8], MD, PhD; Y. Kukida[9], MD, PhD; N. Kondo[10], PhD; Y. Yamano[11], PhD; T. Yanagida[12], MD; K. Endo[13,14], MD; K. Matsui[15], MD, PhD; T. Takeuchi[16], MD, PhD; K. Ichinose[17,18], MD, PhD; M. Kato[19], MD, PhD; R. Yanai[3], MD; Y. Matsuo[20,21], MD, PhD; Y Shimojima[22], MD, PhD; R. Nishioka[23], MD; R. Okazaki[24], MD, PhD; T. Takata[25], MD, PhD; T. Ito[26], MD, PhD; M. Moriyama[18], MD; A. Takatani[27], MD, PhD; Y. Miyawaki[28], MD, PhD; T. Ito-Ihara[29], MD, PhD; T. Kawaguchi[30], PhD; Y. Kawahito[2], MD, PhD; S. Hirata[1], MD, PhD

Affiliations

[1]Department of Clinical Immunology and Rheumatology, Hiroshima University Hospital

[2]Inflammation and Immunology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine

[3]Division of Rheumatology, Department of Medicine, Showa University School of Medicine

[4]Department of Rheumatology, University of Yamanashi Hospital

[5]Department of Internal Medicine and Rheumatology, Juntendo University

[6]Center for Rheumatic Disease, Japanese Red Cross Society Kyoto Daiichi Hospital

[7]Department of Rheumatology, Chubu Rosai Hospital

[8]Immuno-Rheumatology Center, St. Luke's International Hospital

[9]Department of Rheumatology, Japanese Red Cross Society Kyoto Daini Hospital

[10]Department of Nephrology, Kyoto Katsura Hospital

[11]Department of Respiratory Medicine and Allergy, Tosei General Hospital

[12]Department of Hematology and Rheumatology, Kagoshima University Hospital

[13]Department of General internal medicine, Tottori Prefectural Central Hospital

[14]Department of Pharmacoepidemiology, Graduate School of Medicine and Public Health, Kyoto University

[15]Department of Diabetes, Endocrinology and Clinical Immunology, Hyogo Medical University, School of Medicine

[16]Department of Internal Medicine (IV), Osaka Medical and Pharmaceutical University

[17]Department of Immunology and Rheumatology, Division of Advanced Preventive Medical Sciences, Nagasaki University Graduate School of Biomedical Sciences

[18]Department of Rheumatology, Shimane University Faculty of Medicine

[19] Department of Rheumatology, Endocrinology and Nephrology, Graduate School of Medicine, Hokkaido University

[20]Department of Rheumatology, Tokyo Kyosai Hospital

[21]Department of Rheumatology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU)

[22]Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine

[23]Department of Rheumatology, Graduate School of Medical Science, Kanazawa University

[24]Division of Respiratory Medicine and Rheumatology, Department of Multidisciplinary Internal Medicine, Faculty of Medicine, Tottori University

[25]Division of Gastroenterology and Nephrology, Tottori University

[26]Division of Nephrology, Shimane University Hospital

[27]Rheumatic Disease Center, Sasebo Chuo Hospital

[28]Department of Nephrology, Rheumatology, Endocrinology and Metabolism, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences

[29]The Clinical and Translational Research Center, University Hospital, Kyoto Prefectural University of Medicine

[30]Department of Practical Pharmacy, Tokyo University of Pharmacy and Life Sciences

Conflict of interest: S.H. has received speaker's fees, consultancy fees, research grants, or honoraria from AbbVie, Asahi-Kasei, Astellas, Ayumi, Brisytol Myers Squibb, Celgene, Chugai, Eisai, Gilead, Glaxo Smithkline, IIy Lilly, Janssen, Kyorin, Novartis, Sanofi, Tanabe-Mitsubishi, and UCB.

[*]**Corresponding author's name, address, and email:** Yusuke Yoshida

Department of Clinical Immunology and Rheumatology, Hiroshima University Hospital, 1-2-3 Kasumi, Minami, Hiroshima, 734-8551, Japan

Email: fisher37@hiroshima-u.ac.jp

Key Indexing Terms: anti-neutrophil cytoplasmic antibody; anti-neutrophil cytoplasmic antibody-associated vasculitis; eosinophilic granulomatosis with polyangiitis; microscopic polyangiitis; granulomatosis with polyangiitis; seasonal variation

ABSTRACT

Objective: To clarify seasonal and other environmental effects on the onset of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV).

Methods: We enrolled patients with new-onset eosinophilic granulomatosis with polyangiitis (EGPA), microscopic polyangiitis (MPA), and granulomatosis with polyangiitis (GPA) registered in the database of a Japanese multicenter cohort study. We investigated the relationship between environmental factors and clinical characteristics. Seasons were divided into four (spring, summer, autumn, and winter), and the seasonal differences in AAV onset were analyzed using Pearson's chi-squared test, with an expected probability of 25% for each season.

Results: A total of 454 patients were enrolled, with a mean age of 70.9 years and a female proportion of 55.5%. Overall, 74, 291, and 89 patients were classified as EGPA, MPA, and GPA, respectively. Positivity for myeloperoxidase (MPO)-ANCA and proteinase 3 (PR3)-ANCA was observed in 355 and 46 patients, respectively. Overall, the seasonality of AAV onset significantly deviated from the expected 25% for each season ($p=0.001$), and its onset was less frequently observed in autumn. In ANCA serotypes, seasonality was significant in patients with MPO-ANCA ($p<0.001$), but not in those with PR3-ANCA ($p=0.97$). Additionally, rural residency of patients with AAV was associated with PR3-ANCA positivity and biopsy-proven pulmonary vasculitis.

Conclusion: The onset of AAV was influenced by seasonal variations and was less frequently observed in autumn. In contrast, the occurrence of PR3-ANCA was not triggered by season, but by rural residency.

Introduction

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a major disease entity of systemic necrotizing vasculitis that predominantly affects the small vessels. AAV is classified into the following three subtypes: eosinophilic granulomatosis with polyangiitis (EGPA), microscopic polyangiitis (MPA), and granulomatosis with polyangiitis (GPA), according to the definition by the Chapel Hill Consensus Conference 2012 (CHCC2012) (1). The etiology and pathogenesis of AAV are yet to be fully elucidated; however, several environmental factors can trigger the formation of neutrophil extracellular traps (NETs), leading to the production of autoantibodies against released neutrophil cytoplasmic autoantigens. ANCAs against these autoantigens, including myeloperoxidase (MPO) and proteinase 3 (PR3), contribute to excessive neutrophil activation and NET formation, resulting in the development of AAV (2). One of the well-known triggering environmental factors of AAV is the use of drugs such as propylthiouracil (anti-thyroid drug) and hydralazine (anti-hypertensive drug), which induce NET formation; therefore, this condition is defined as drug-associated AAV (3).

Furthermore, seasonal influences on AAV development have been investigated. Among the seasonality-based cohorts, the four largest case numbers were 138 (AAV, 91; renal limited vasculitis, 36; other types of small-vessel vasculitis, 11), 234, 339, and 445 conducted in Örebro,

Sweden (4); Catalonia, Spain (5); Scotland, United Kingdom (6); and Hamburg, Germany (7), respectively. In Scottish populations with MPA or GPA, the seasonality for the time of diagnosis was not observed (6); however, it is difficult to interpret seasonal effect on AAV because AAV diagnosis is often challenging and the duration from onset to diagnosis can be influenced by the patient's or doctor's delay. Additionally, in an investigation targeting a German population with GPA, no seasonal variation was observed (7) and the remaining two cohorts suggested that the frequency of AAV onset with renal involvement was high in the winter (4,5). In a study conducted in Japan, there was only a single cohort regarding the seasonality of AAV, and 56 patients with AAV were enrolled; however, this analysis only considered the seasonality of the time of diagnosis (8). Furthermore, there are several reports focused on the association of seasons with AAV from different points of view. Among them, increased ANCA in autumn was associated with the relapse of AAV (9), and the hospitalization of patients with AAV was most observed in winter (10).

Additionally, smoking habit or advanced age may influence the incidence of AAV, although it is still unclear whether smoking protects against the development of AAV (11,12). Other environmental factors such as geographic location may also contribute to the incidence of AAV (6,8,13).

It is important to elucidate environmental effects on the development of AAV to better understand the pathogenesis of AAV and prevent its development. Therefore, in this study, we analyzed the data collected from a multicenter study conducted in Japan (Japan Collaborative Registry of ANCA-Associated Vasculitis [J-CANVAS]) to clarify the seasonal and other environmental effects on the onset of AAV.

Methods

Study design

This was a retrospective cohort study using the information collected from J-CANVAS conducted at 24 institutions across Japan from the Kyushu area to Hokkaido area. We investigated the relationship between environmental factors, including seasonal and geographical variations, and clinical characteristics of the patients with AAV, such as the disease type, ANCA serotype, and organ involvement.

Patients and definition

Patients who were registered in the J-CANVAS study and had newly developed AAV between January 2017 and December 2019 were enrolled in this study. We excluded patients from the analyses of seasonality whose information regarding the month of the onset of AAV was not available. All patients were aged ≥20 years, and AAV was classified into EGPA, MPA, and GPA

based on the definition of CHCC2012 (1). AAV onset was defined as the first awareness of general symptoms or organ-specific findings suggesting AAV, such as fever, arthralgia, myalgia, weight loss, and abnormalities of the ear, eye, nose, throat, skin, lungs, kidneys, and nerves, based on the Birmingham Vasculitis Activity Score version 3 (14).

Data collection

Patient information such as age at the time of diagnosis, the time of onset and diagnosis, biopsy results of the kidney or lung, smoking habit status, and ANCA profiles were available. All extracted information from medical records were obtained using an electric data capture system, Viedoc (PCG Solutions, Uppsala, Sweden).

In the analyses of seasonality, seasons were defined according to the Japanese Meteorological Agency (JMA) as spring (March–May), summer (June–August), autumn (September–November), and winter (December–February) (15). In geographical analyses, the residential areas of the patients were confirmed by postal cord information, and the population density was determined according to published data released by the Japanese Ministry of Internal Affairs and Communications in 2020 (16). Additionally, rural areas are defined as those with a population density of <200 persons per square kilometer and urban areas as ≥200 persons per square kilometer. The climatic zones of Japan were classified into the following eleven zones based on the definition of the JMA (15): Hokkaido, Tohoku, Kanto/Kosin, Tokai, Hokuriku, Kinki,

Chugoku, Shikoku, Kyushu (North), Kyushu (South) and Amami, and Okinawa.

ANCAs were detected by indirect immunofluorescence (IIF) or enzyme immunoassay (EIA). In the results of this study, positive results for p-ANCA in IIF and MPO-ANCA in EIA were both described as positive for MPO-ANCA. Similarly, c-ANCA and PR3-ANCA were described as PR3-ANCA.

This study was approved by the Ethical Committee for Epidemiology of Hiroshima University (approval number: E-2021-2465), and performed in accordance with the ethical standards of the Helsinki Declaration. The requirement for written consent was waived because of the retrospective nature of the study.

Statistical analysis

Descriptive statistics are presented as mean ± standard deviation, or median (interquartile range) for continuous variables, and as absolute numbers (percentage) for categorical variables. A non-parametric Kruskal-Wallis test was applied for the comparison of numerical variables, and the Fisher's exact test or Pearson's chi-squared test was used for categorical variables in the analyses of baseline characteristics. In the seasonal analyses, the seasonal differences were investigated if the AAV onset in each season was expected to be 25% using the Pearson's chi-squared test. The analysis for the association of ANCA and environmental factors was performed using the Fisher's exact test. Logistic regression models were used to determine the predictors of organ involvement

in patients with biopsy-proven vasculitis, adjusted by patient age and the season of onset. Statistical significance was set at $p<0.05$. All statistical analyses were performed using JMP Pro 16 (SAS Institute Inc., San Diego, CA, USA).

Results

Baseline characteristics of patients with AAV

A total of 454 patients with new-onset AAV were enrolled in this study. All of the patients were of Asian ethnicity. Among these, 74 were classified into EGPA, 291 into MPA, and 89 into GPA, with a mean age of 60.7 ± 13.9, 73.6 ± 11.6, and 70.2 ± 13.2 years, and a female proportion of 55.4%, 55.7%, and 55.1%, respectively, as described in Table 1. The mean duration from the onset of EGPA, MPA, and GPA to diagnosis was 51.8 ± 53.0, 82.8 ± 94.3, and 97.7 ± 96.8 days, respectively. Table 1 summarizes the detailed characteristics of the three types of AAV. Biopsy-proven vasculitis of the kidneys was observed in no patients with EGPA, 39.5% with MPA, and 27.0% with GPA, whereas that of the lungs was observed in 2.7% of patients with EGPA, 1.4% with MPA, and 14.6% with GPA. The profiles of smoking habits were comparable among the three groups ($p=0.66$).

Baseline ANCA serotypes of patients with AAV

MPO-ANCA positivity was found in 33.8% of patients with EGPA, 95.9% with MPA, and 57.3%

with GPA, whereas that of PR3-ANCA was found in 2.7% of patients with EGPA, 3.1% with MPA, and 39.3% with GPA. High prevalence of MPO-ANCA in patients with GPA seems to reflect the characteristics of Japanese population with AAV (17).

Seasonal differences of the onset of AAV

Spring onset was observed in 28.4% of the enrolled patients with new-onset AAV, summer in 28.9%, autumn in 17.2%, and winter in 25.6%. The seasonality of the onset significantly deviated ($p=0.01$) from the expected 25% for each season, as shown in Table 2 and Figure 1A. The distributions of seasonal number of patients were comparable between 2017, 2018 and 2019 (Figure 1B). Subsequently, we analyzed seasonality in terms of disease types (Figure 1C,1D). In patients with MPA, it was 31.3%, 27.9%, 15.8%, and 25.1%, in spring, summer, autumn, and winter, respectively, and marked seasonality was observed ($p<0.001$). However, in patients with EGPA, spring onset was observed in 28.4%, summer onset in 21.6%, autumn onset in 23.0%, and winter onset in 27.0%, and the seasonal difference was not significant ($p=0.92$). Moreover, it was not significant in GPA (27.0%, 23.6%, 16.9%, and 32.6%, in spring, summer, autumn, and winter, respectively, $p=0.20$).

Next, we investigated the seasonality stratified by age (Figure 2). Among the enrolled patients with AAV aged ≥65 years, the frequency of autumn onset was low in every disease type, while

in those aged <65 years, the frequency of spring onset was high in EGPA or MPA, but not in GPA. Moreover, among the three disease types of AAV, significant seasonality was observed in patients with MPA aged ≥65 years (p=0.002) and in those aged <65 years (p=0.02) while not in EGPA and GPA.

Association between ANCA and other environmental factors

In the results of the seasonal analyses, among patients with MPO-ANCA, 29.3% developed AAV in spring, 30.7% in summer, 15.8% in autumn, and 24.2% in winter (Table 2), and the seasonal difference was significant (p<0.001). However it was not observed in patients with PR3-ANCA (21.7%, 26.1%, 26.1%, and 26.1%, in spring, summer, autumn, and winter, respectively, p=0.97), indicating that the onset of AAV in patients with PR3-ANCA was not affected by the season. Next, we investigated the triggering factors of AAV, including smoking habits and rurality. We obtained information on the smoking status of 432 patients. Among 203 patients who smoked (current or past smoker), 153 were positive for MPO-ANCA, 22 were positive for PR3-ANCA, and the remaining 28 were negative for ANCA. These proportions were comparable to 229 patients of never-smokers (184 positive for MPO-ANCA, 22 positive for PR3-ANCA, and 23 negative for ANCA) (p=0.41). In addition, we performed geographical analyses of 396 patients with AAV for whom postal cord information was available. Among 321 patients who lived in

urban areas, 254 were positive for MPO-ANCA, 28 were positive for PR3-ANCA, and 39 were negative for ANCA. These proportions were significantly different from 75 patients living in rural areas (MPO-ANCA, 62; PR3-ANCA, 11; ANCA-negative, 2) (p=0.01), suggesting that the presence of PR3-ANCA is associated with rurality.

Association of geographical location with the season of onset and organ involvements in AAV

The climatic zones were classified into the eleven zones based on the definition provided by JMA. Of the 403 eligible patients with AAV, eight belonged to Hokkaido, 110 to Kanto/Kosin, five to Tokai, 21 to Hokuriku, 137 to Kinki, 66 to Chugoku, 40 to Kyushu (North), 16 to Kyushu (South) and Amami zones, and none belonged to Tohoku, Shikoku and Okinawa zones. Of note, autumn onset had the lowest incidence in all zones where more than 10 patients of AAV were living (Figure 3). Finally, we investigated the association between the geographical location and organ involvement in AAV. Among 135 patients who underwent kidney biopsy, the proportion of biopsy-proven vasculitis was not different between patients living in rural areas and those living in urban areas (91.4% vs. 92.0%, Table 3). However, among the 47 patients who underwent lung biopsy, the proportion of biopsy-proven vasculitis in patients living in rural areas was significantly different from that in those living in urban areas after adjusting for age and season [66.7% vs. 31.6%; odds ratio (95% confidence interval), 10.07 (1.48–68.42); p=0.02].

Discussion

We conducted this study to identify the environmental factors for the development of AAV or ANCA, focusing on seasonal variations in Japan. The results of our study suggest that the onset of AAV is less frequently observed in autumn; however, in the analyses by disease subtype, the onset of EGPA and GPA was not associated with seasonal changes. In addition, patients with PR3-ANCA develop AAV without any relation to seasonal influence, but with rural residency, which is associated with the formation of vasculitis in the lungs.

The triggers of AAV may vary by season. For example, they might be bacteria causing respiratory infections in winter and antigens causing allergy-related diseases in spring or summer (11). Serum levels of vitamin D, whose active form acts as an immunomodulator, are highest in late summer and lowest in late winter. A lower incidence of vitamin D deficiency was suggested in autumn (9,11). These factors can be related to the lower incidence of AAV in autumn, although we do not know why it was especially observed in MPO-ANCA-positive AAV in our study.

Interestingly, the results of our study regarding non-seasonality of the onset of GPA was the same as that of the largest previous cohort targeting GPA, reporting that the onset of GPA is not associated with seasonal variation (7). This is consistent with our result that PR3-ANCA was not associated with seasonality, as most of the patients with PR3-ANCA develop GPA. Japanese

patients with MPO-ANCA often develop GPA (17), which may explain why no seasonality was clear in patients with PR3-ANCA, but less so with GPA in our study.

In contrast, previous research investigating environmental factors has suggested that exposure to specific inhaled antigens such as silica and grain dust may be a risk factor for AAV (18-20). GPA predominantly shows involvement of the upper and lower respiratory tracts at diagnosis (21), indicating that environmental factors such as inhaled antigens induce the development of granuloma and vasculitis through the aberrant activation of neutrophils and NET formation (2). This appears to be related to our finding that biopsy-proven pulmonary vasculitis, which is not commonly observed in AAV (except GPA or PR3-ANCA-positive AAV), is often observed in patients with AAV living in rural areas. In addition, our data revealed that biopsy-proven renal vasculitis of AAV was observed equally in both rural and urban areas while it was previously considered predominant in rural areas based on the results of data analyses of 33 patients with AAV conducted in Canada (13).

There are some limitations associated with our study, because our analyses used the pooled cohort data. First, we could not obtain information regarding ANCA titers measured by EIA; therefore, analyses of the relationship between ANCA titers and seasonality or living areas could not be performed. Besides, information regarding the drug intake of patients before the onset of AAV was also not available; therefore, we were not able to investigate the association of the

development of AAV and drug exposure. Second, all enrolled patients were of Asian descent; however, we could not identify further details (whether or not they were Japanese). The likelihood to develop any disease subtype or any ANCA serotype can be influenced by ethnicity (22); therefore, its stratification was preferred for the analysis of the association between AAV and environmental factors. However, Japan is not originally a multi-ethnic country; therefore, it is expected that there were not many people with ethnicities other than Japanese. Third, we found an association between inhaled antigens related to rurality and vasculitis formation in patients with GPA or PR3-ANCA; however, further exploration of the pathogen cannot be performed due to the nature of this study.

The strength of our study is that it has the largest number of cases of any study in which seasonal and other environmental effects on the onset of AAV have been investigated to date.

In conclusion, the development of AAV is influenced by seasonal variations and the seasonality depends on the ANCA serotype. Furthermore, the occurrence of PR3-ANCA is not triggered by seasonal variations, but by rural residency.

Acknowledgments

We thank all staff who treated the enrolled patients at the collaborating institutions. We also thank S. Masuda and A. Yorishima (Hiroshima Prefectural Hospital, Hiroshima, Japan) for helping us

collect the information of patients with AAV at our institution.

References

1. Jennette JC, Falk RJ, Bacon PA, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum 2013;65:1-11.

2. Nakazawa D, Masuda S, Tamaru U, Ishizu A. Pathogenesis and therapeutic interventions for ANCA-associated vasculitis. Nat Rev Rheumatol 2019;15:91-101.

3. Arneth B, Arneth R. Neutrophil extracellular traps (Nets) and vasculitis. Int J Med Sci 2021;18:1532-40.

4. Tidman M, Olander R, Svalander C, Danielsson D. Patients hospitalized because of small vessel vasculitides with renal involvement in the period 1975-95: organ involvement, anti-neutrophil cytoplasmic antibodies patterns, seasonal attack rates and fluctuation of annual frequencies. J Intern Med 1998;244:133-41.

5. Draibe J, Rodó X, Fulladosa X, et al. Seasonal variations in the onset of positive and negative renal ANCA-associated vasculitis in Spain. Clin Kidney J 2018;11:468-73.

6. Aiyegbusi O, Frleta-Gilchrist M, Traynor JP, et al. ANCA-associated renal vasculitis is associated with rurality but not seasonality or deprivation in a complete national cohort study. RMD Open 2021;7:e001555.

7. Aries PM, Herlyn K, Reinhold-Keller E, Latza U. No seasonal variation in the onset of symptoms of 445 patients with Wegener's granulomatosis. Arthritis Rheum 2008;59:904.

8. Fujimoto S, Uezono S, Hisanaga S, et al. Incidence of ANCA-associated primary renal vasculitis in the Miyazaki Prefecture: the first population-based, retrospective, epidemiologic survey in Japan. Clin J Am Soc Nephrol 2006;1:1016-22.

9. Kemna MJ, Cohen Tervaert JW, Broen K, Timmermans SAMEG, van Paassen P, Damoiseaux JGMC. Seasonal influence on the risk of relapse at a rise of antineutrophil cytoplasmic antibodies in vasculitis patients with renal involvement. J Rheumatol 2017;44:473-81.

10. Li J, Cui Z, Long JY, Huang W, Wang JW, Wang H. The frequency of ANCA-associated vasculitis in a national database of hospitalized patients in China. Arthritis Res Ther 2018;20:226.

11. Zhao WM, Wang ZJ, Shi R, et al. Environmental factors influencing the risk of ANCA-associated vasculitis. Front Immunol 2022;13:991256.

12. Maritati F, Peyronel F, Fenaroli P, et al. Occupational Exposures and Smoking in Eosinophilic Granulomatosis With Polyangiitis: A Case-Control Study. Arthritis Rheumatol 2021;73:1694-702.

13. Anderson K, Klassen J, Stewart SA, Taylor-Gjevre RM. Does geographic location affect incidence of ANCA-associated renal vasculitis in northern Saskatchewan, Canada? Rheumatology (Oxford) 2013;52:1840-4.

14. Mukhtyar C, Lee R, Brown D, et al. Modification and validation of the Birmingham Vasculitis Activity Score (version 3). Ann Rheum Dis 2009;68:1827-32.

15. Overview of Japan's climate. [Internet. Accessed January 05, 2023] Available from: https://www.data.jma.go.jp/gmd/cpd/longfcst/en/tourist_japan.html

16. Statistics of Japan. [Internet. Accessed January 05, 2023] Available from: https://www.e-stat.go.jp/en/stat-search/files?page=1&layout=datalist&toukei=00200502&tstat=000001166301&cycle=0&tclass1=000001166302&cycle_facet=tclass1&tclass2val=0

17. Hara A, Wada T, Sada KE, et al. Risk Factors for Relapse of Antineutrophil Cytoplasmic Antibody-associated Vasculitis in Japan: A Nationwide, Prospective Cohort Study. J Rheumatol 2018;45:521-8.

18. Lane SE, Watts RA, Bentham G, Innes NJ, Scott DG. Are environmental factors important in primary systemic vasculitis? A case-control study. Arthritis Rheum 2003;48:814-23.

19. Gómez-Puerta JA, Gedmintas L, Costenbader KH. The association between silica exposure and development of ANCA-associated vasculitis: systemic review and meta-analysis. Autoimmunity Rev 2013;12:1129-35.

20. Stamp LK, Chapman PT, Francis J, et al. Association between environmental exposures

and granulomatosis with polyangiitis in Canterbury, New Zealand. Arthritis Res Ther 2015;17:333.

21. Abdou NI, Kullman GJ, Hoffman GS, et al. Wegener's granulomatosis: survey of 701 patients in North America. Changes in outcome in the 1990s. J Rheumatol 2002;29:309-16.

22. Trivioli G, Marquez A, Martorana D, et al. Genetics of ANCA-associated vasculitis: role in pathogenesis, classification and management. Nat Rev Rheumatol 2022;18:559-74.

Figure Legends

Figure 1

(**A**) The seasonal variations of the onset of anti-neutrophil cytoplasmic antibody-associated vasculitis (AAV). (**B**) The distribution of seasonal number of patients with AAV in 2017, 2018, and 2019. (**C**) The distribution of seasons of the onset of eosinophilic granulomatosis with polyangiitis (EGPA), microscopic polyangiitis (MPA), and granulomatosis with polyangiitis (GPA). (**D**) The distribution of months (JAN: January, FEB: February, MAR: March, APR: April, MAY: May, JUN: June, JUL: July, AUG: August, SEP: September, OCT: October, NOV: November, DEC: December) of the onset of EGPA, MPA, and GPA.

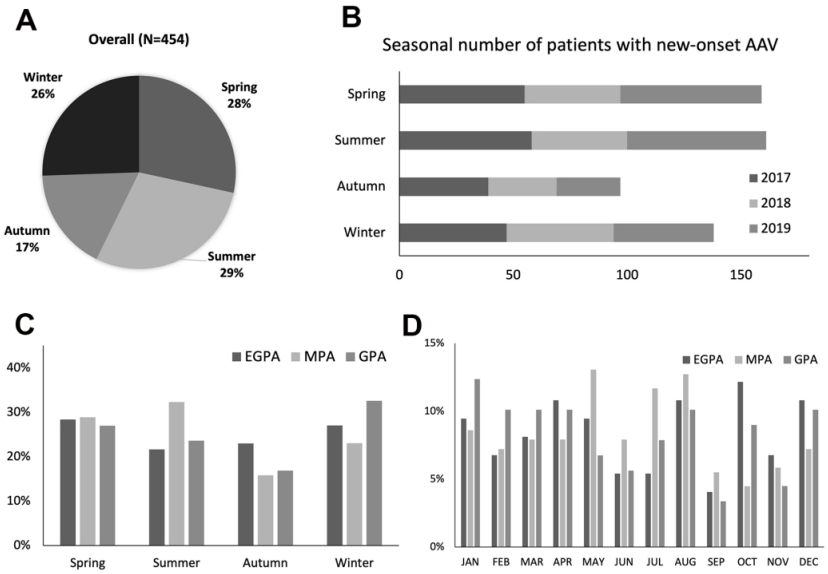
Figure 2

The seasonal variations of the onset of each anti-neutrophil cytoplasmic antibody-associated vasculitis stratified by age (≥65 years or less). The distribution of seasons of the onset of eosinophilic granulomatosis with polyangiitis (EGPA), microscopic polyangiitis (MPA), and granulomatosis with polyangiitis (GPA).

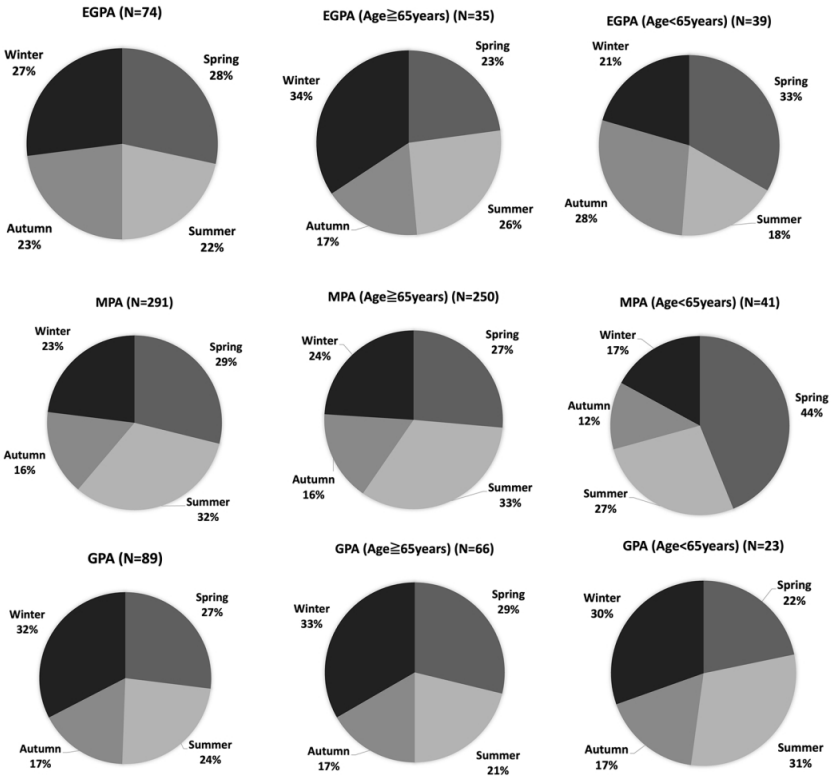
Figure 3

Classification of the climatic zones of Japan into the following eleven zones based on the

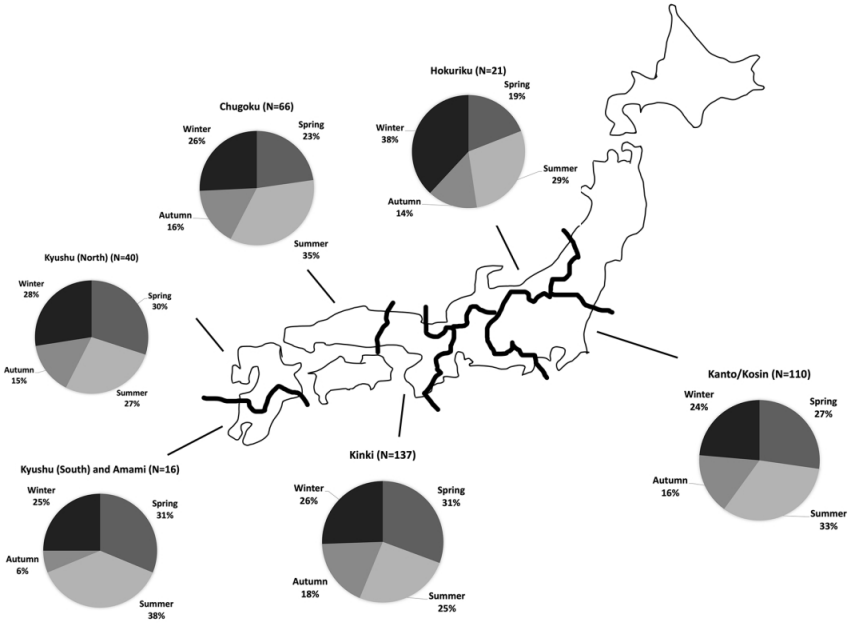
definition of the Japanese Meteorological Agency: Hokkaido, Tohoku, Kanto/Kosin, Tokai, Hokuriku, Kinki, Chugoku, Shikoku, Kyushu (North), Kyushu (South) and Amami, and Okinawa. The seasonality of the onset of anti-neutrophil cytoplasmic antibody-associated vasculitis are described only for zones populated by more than 10 patients.



174x118mm (300 x 300 DPI)



151x141mm (300 x 300 DPI)



167x120mm (300 x 300 DPI)

Table 1 Baseline demographics of the new-onset AAV

Variables		*Overall (N=454)*	*EGPA (N=74)*	*MPA(N=291)*	*GPA(N=89)*	*P value (EGPA vs MPA vs GPA)*
Age (years)		70.9 ± 13.2, 74.0 (66.0 – 80.0)	60.7 ± 13.9, 62.5 (49.8 – 70.3)	73.6 ± 11.6, 76.0 (69.0 – 82.0)	70.2 ± 13.2, 73.0 (64.0 – 78.5)	<0.001*
Patients aged ≥65 years		351 (77.3)	35 (47.3)	250 (85.9)	66 (74.2)	<0.001*
Female		252 (55.5)	41 (55.4)	162 (55.7)	49 (55.1)	0.99
Disease duration from the onset of AAV to the diagnosis (days)		80.9 ± 90.5, 49.0 (23.0 – 100.0)	51.8 ± 53.0, 29.0 (17.5 – 71.5)	82.8 ± 94.3, 49.0 (26.0 – 99.0)	97.7 ± 96.8, 62.0 (32.0 – 131.0)	0.001*
Biopsy-proven vasculitis in organ involvement	Kidney	139 (30.6)	0 (0)	115 (39.5)	24 (27.0)	<0.001*
	Lung	19 (4.2)	2 (2.7)	4 (1.4)	13 (14.6)	<0.001*
Smoking habit	Never	229 (50.4)	36 (48.7)	149 (51.2)	44 (49.4)	0.66
	Past	165 (36.3)	29 (39.2)	107 (36.8)	29 (32.6)	
	Current	38 (8.4)	6 (8.1)	20 (6.9)	12 (13.5)	
	Unknown	22 (4.8)	3 (4.1)	15 (5.2)	4 (4.5)	
Serotype of ANCA	Positivity for MPO-ANCA	355 (78.2)	25 (33.8)	279 (95.9)	51 (57.3)	<0.001*

Positivity for PR3-ANCA	46 (10.1)	2 (2.7)	9 (3.1)	35 (39.3)
Double negativity	53 (11.7)	47 (63.5)	3 (1.0)	3 (3.4)

*<0.05

Values are mean ± standard deviation, median (interquartile range) or number (%)

Continuous variables are compared by the Kruskal-Wallis test. Categorical variables are compared by Fisher's exact test or chi-square test.

AAV ANCA-associated vasculitis, *EGPA* eosinophilic granulomatosis with polyangiitis, *MPA* microscopic polyangiitis, *GPA* granulomatosis with polyangiitis, *ANCA* anti-neutrophil cytoplasmic antibody, *MPO* myeloperoxidase, *PR3* proteinase-3

Table 1 Baseline demographics of the new-onset AAV

Variables		*Overall (N=454)*	*EGPA (N=74)*	*MPA(N=291)*	*GPA(N=89)*	*P value (EGPA vs MPA vs GPA)*
Age (years)		70.9 ± 13.2, 74.0 (66.0 – 80.0)	60.7 ± 13.9, 62.5 (49.8 – 70.3)	73.6 ± 11.6, 76.0 (69.0 – 82.0)	70.2 ± 13.2, 73.0 (64.0 – 78.5)	<0.001*
Patients aged ≥65 years		351 (77.3)	35 (47.3)	250 (85.9)	66 (74.2)	<0.001*
Female		252 (55.5)	41 (55.4)	162 (55.7)	49 (55.1)	0.99
Disease duration from the onset of AAV to the diagnosis (days)		80.9 ± 90.5, 49.0 (23.0 – 100.0)	51.8 ± 53.0, 29.0 (17.5 – 71.5)	82.8 ± 94.3, 49.0 (26.0 – 99.0)	97.7 ± 96.8, 62.0 (32.0 – 131.0)	0.001*
Biopsy-proven vasculitis in organ involvement	Kidney	139 (30.6)	0 (0)	115 (39.5)	24 (27.0)	<0.001*
	Lung	19 (4.2)	2 (2.7)	4 (1.4)	13 (14.6)	<0.001*
Smoking habit	Never	229 (50.4)	36 (48.7)	149 (51.2)	44 (49.4)	0.66
	Past	165 (36.3)	29 (39.2)	107 (36.8)	29 (32.6)	
	Current	38 (8.4)	6 (8.1)	20 (6.9)	12 (13.5)	
	Unknown	22 (4.8)	3 (4.1)	15 (5.2)	4 (4.5)	
Serotype of ANCA	Positivity for MPO-ANCA	355 (78.2)	25 (33.8)	279 (95.9)	51 (57.3)	<0.001*

Positivity for PR3-ANCA	46 (10.1)	2 (2.7)	9 (3.1)	35 (39.3)
Double negativity	53 (11.7)	47 (63.5)	3 (1.0)	3 (3.4)

*<0.05

Values are mean ± standard deviation, median (interquartile range) or number (%)

Continuous variables are compared by the Kruskal-Wallis test. Categorical variables are compared by Fisher's exact test or chi-square test.

AAV ANCA-associated vasculitis, *EGPA* eosinophilic granulomatosis with polyangiitis, *MPA* microscopic polyangiitis, *GPA* granulomatosis with polyangiitis, *ANCA* anti-neutrophil cytoplasmic antibody, *MPO* myeloperoxidase, *PR3* proteinase-3

Table 2 Association between ANCA serotype and environmental factors

	ANCA serotype of patients with AAV			
	Overall	***MPO-ANCA***	***PR3-ANCA***	***Double negativity***
Seasonality of the onset	*N=454*	*N=355*	*N=46*	*N=53*
Spring	129 (28.4)	104 (29.3)	10 (21.7)	15 (28.3)
Summer	131 (28.9)	109 (30.7)	12 (26.1)	10 (18.9)
Autumn	78 (17.2)	56 (15.8)	12 (26.1)	10 (18.9)
Winter	116 (25.6)	86 (24.2)	12 (26.1)	18 (34.0)
Status of smoking habit	N=432	N=337	N=44	N=51
Never	229 (53.0)	184 (54.6)	22 (50.0)	23 (45.1)
Current or past	203 (47.0)	153 (45.4)	22 (50.0)	28 (54.9)
Living area	N=396	N=316	N=39	N=41
Rural	75 (18.9)	62 (19.6)	11 (28.2)	2 (4.9)
Urban	321 (81.1)	254 (80.4)	28 (71.8)	39 (95.1)

Values are number (%).

The seasons of spring, summer, autumn and winter are defined according to the definition of Japanese Meteorological Agency.

AAV ANCA-associated vasculitis, *ANCA* anti-neutrophil cytoplasmic antibody, *MPO* myeloperoxidase, *PR3* proteinase-3

Table 3 Association of geographical location and organ involvements

		geographical location	
	Overall	*Rural area*	*Urban area*
Patients who underwent kidney biopsy	135	35	100
Biopsy-proven vasculitis	124 (91.9)	32 (91.4)	92 (92.0)
No evidence of vasculitis	11 (8.1)	3 (8.6)	8 (8.0)
Odds ratio (95% confidence interval)		0.98 (0.24 – 4.04), p=0.98	reference
Patients who underwent lung biopsy	47	9	38
Biopsy-proven vasculitis	18 (38.3)	6 (66.7)	12 (31.6)
No evidence of vasculitis	29 (61.7)	3 (33.3)	26 (68.4)
Odds ratio (95% confidence interval)		10.07 (1.48 – 68.42), p=0.02*	reference

Values are number (%). *<0.05

AAV ANCA-associated vasculitis

Multivariable logistic analyses are performed by adjusting age (age ≥65 years old or less) and season.

Rural areas are defined as those with a population density of <200 person per square kilometer, and urban areas as ≥200 persons per square kilometer.