The Journal of Rheumatology 2023;50:330–4
doi:10.3899/jrheum.220526
First Release December 1 2022

The Journal of Rheumatology

Effects of Aging on Rheumatoid Factor and Anticyclic Citrullinated Peptide Antibody Positivity in Patients With Rheumatoid Arthritis

Satoshi Takanashi[1], Tsutomu Takeuchi[1], and Yuko Kaneko[1]

ABSTRACT. ***Objective.*** We investigated the factors that affect rheumatoid factor (RF) and anticyclic citrullinated peptide antibody (anti-CCP) positivity in patients with rheumatoid arthritis (RA).

Methods. The study included all consecutive patients with RA treated at Keio University Hospital between 2016 and 2017. We recorded age at diagnosis, sex, smoking habit, BMI (kg/m^2), and family history, and investigated the association between these variables and RF and anti-CCP positivity.

Results. We recruited 1685 patients with RA. The mean age at diagnosis was 51.9 years, and 83.4% of the patients were women. Positivity rates of RF and anti-CCP almost linearly decreased along with the increase in age at RA diagnosis (grouped by decade) after ≥ 30 years of age (RF: 80.5%, 84.2%, 81.1%, 78%, 74.6%, 62.6%, 51.4%, $P < 0.001$; anti-CCP: 79.9%, 87.4%, 81.7%, 74%, 70.5%, 60.2%, 37.1%; $P < 0.001$). Multivariable analysis revealed that age was independently associated with seronegativity in women (RF: odds ratio [OR] 0.98, 95% CI 0.97-0.99, $P < 0.001$; anti-CCP: OR 0.97, 95% CI 0.96-0.98; $P < 0.001$), nonsmoking history (RF: OR 0.98, 95% CI 0.97-0.99, $P < 0.001$; anti-CCP: OR 0.97, 95% CI 0.96-0.98; $P < 0.001$), and BMI < 25 (RF: OR 0.98, 95% CI 0.97-0.99; $P < 0.001$; anti-CCP: OR 0.97, 95% CI 0.97-0.98; $P < 0.001$).

Conclusion. Aging is an independent contributor for seronegative RA in patients who are female, have a nonsmoking history, and a BMI < 25.

Key Indexing Terms: aging, anticyclic citrullinated peptide, elderly-onset rheumatoid arthritis, rheumatoid arthritis, rheumatoid factor, seronegative

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by synovitis that leads to bone destruction and functional disability.[1] Rheumatoid factor (RF) and anticyclic citrullinated peptide antibodies (anti-CCP) are serological hallmarks of RA, and these autoantibodies were included in the classification criteria proposed by the American College of Rheumatology (ACR) and European Alliance of Associations for Rheumatology (EULAR) in 2010.[2,3] Autoantibody production is implicated in the pathogenesis of RA and is attributable to several factors that may be categorized into genetic contributors, such as shared epitopes, and environmental contributors, such as cigarette smoking and periodontal disease.[1]

RF and anti-CCP are usually positive in 70% to 80% of patients with RA; however, a recent study has reported an increase in the number of patients with seronegative RA encountered in clinical practice.[4] The increase in prevalence of seronegative RA is attributed to lifestyle changes, including reduced smoking and obesity[4]; however, a cohort study that investigated the association between the recent increase in elderly-onset RA and its serology implicates aging as a potential contributor.[5] An aging society warrants greater attention to the increasing prevalence of elderly-onset RA because it differs from young-onset RA with regard to clinical and laboratory features.[6,7] It is important to identify factors associated with RF and anti-CCP positivity, particularly the role of age, for accurate diagnosis and establishment of an optimal treatment strategy; however, to date, few reports have discussed this subject.

In this study, we investigated the factors associated with autoantibody production in patients with RA.

METHODS

Patients. We enrolled consecutive patients with RA, diagnosed based on the 1987 ACR[8] or the 2010 ACR/EULAR criteria,[2] who visited Keio University Hospital between 2016 and 2017. Patients with insufficient data were excluded from the study. This study was approved by the Ethics Committee of the Keio University School of Medicine (approval number: 20130506) and was performed in accordance with the principles of the Declaration of Helsinki. Written informed consent was waived in accordance with Japanese regulations.

Data collection and definitions. We recorded the following clinical data at the time of diagnosis from patients' medical records: sex, age, smoking status, BMI (calculated as weight in kilograms divided by height in meters squared), family history of RA, and serum RF and anti-CCP positivity. We

[1]*S. Takanashi, MD, PhD, T. Takeuchi, MD, PhD, Y. Kaneko, MD, PhD, Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan.*

The authors declare no conflicts of interest relevant to this article.

Address correspondence to Dr. Y. Kaneko, Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.
Email: ykaneko.z6@keio.jp.

Accepted for publication October 17, 2022.

also examined Steinbrocker stage for radiographical assessment[9] and the presence of interstitial lung disease (ILD) during the disease course. The presence of ILD was assessed with plain chest radiographs in all patients, with further checkups with high-resolution computed tomography findings in patients who had pulmonary symptoms or abnormal findings in the chest radiographs. We defined smoking history as a current or past smoking habit at the time of RA diagnosis. Serum RF and anti-CCP levels were measured using a commercially available latex photometric immunoassay system kit (LZ test, Eiken) for RF, which detects IgM-RF, and a chemiluminescent immunoassay kit for anti-CCP (Abbott). Anti-CCP positivity in patients who were not screened for anti-CCP at the time of diagnosis (for example, before the anti-CCP kit was available) was substituted with results obtained on testing during the disease course. We defined a family history of RA as patients with a history of RA in first- or second-degree relatives. Overweight was defined according to the World Health Organization classification as a BMI ≥ 25.

Statistical analysis. Continuous variables are presented as the mean and SD and were compared using the *t* test. Categorical variables are presented as percentages and were compared using the Fisher exact test. The Cochran-Armitage test was used to test the trend. Covariates that were identified as potentially significant factors on univariable analysis were subjected to multivariable logistic regression analysis using a binomial model. A *P* value < 0.05 was considered statistically significant. All statistical analyses were performed using EZR software (version 1.4; https://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html).[10]

RESULTS

Patients and clinical characteristics. We enrolled 2044 patients with RA; after excluding 359 patients with insufficient data, 1685 patients were included in the study. The Table summarizes the clinical characteristics of patients diagnosed with RA. The mean age at diagnosis was 51.9 (SD 15.4) years, and 83.4% of the patients were women. RF and anti-CCP positivity were observed in 76.8% and 74.9% of patients, respectively. RA was diagnosed in 169 (10%) patients aged < 30 years, in 215 (12.8%) patients in their 30s, in 322 (19.1%) patients in their 40s, in 427 (25.3%) patients in their 50s, in 346 (20.5%) patients in their 60s, in 171 (10.1%) patients in their 70s, and in 35 (2.1%) patients ≥ 80 years of age. In this study, 24.9% of patients had a history of smoking, 9.6% had a family history of RA, and 21.5% of patients were overweight. Rate of Steinbrocker stage ≥ 2 and presence of ILD were higher in seropositive patients, but most of the seronegative patients had bone destruction characteristics for RA or ILD as well, suggesting that diagnosis of seronegative RA was valid.

Patients were categorized based on the detection of RF or anti-CCP antibodies; we observed significant intergroup differences in age, sex, BMI, and a family history of RA (Table).

Factors associated with seropositivity. Binomial logistic regression analysis that included data of all patients revealed that age (RF: odds ratio [OR] 0.98, 95% CI 0.97-0.99, $P < 0.001$; anti-CCP: OR 0.98, 95% CI 0.97-0.99, $P < 0.001$), sex (RF: OR 2.12, 95% CI 1.50-3.01, $P < 0.001$; anti-CCP: OR 2.66, 95% CI 1.86-3.72, $P < 0.001$), and a history of smoking (RF: OR 1.62, 95% CI 1.16-2.26, $P = 0.005$; anti-CCP: OR 1.73, 95% CI 1.24-2.42, $P = 0.001$) were independently associated with both RF and anti-CCP positivity (Supplementary Table S1, available with the online version of this article).

Analysis after sex-based subcategorization of patients showed that in women, age (RF: OR 0.98, 95% CI 0.97-0.99, $P < 0.001$; anti-CCP: OR 0.97, 95% CI 0.96-0.98, $P < 0.001$) and a history of smoking (anti-CCP: OR 1.63, 95% CI 1.08-2.47, $P = 0.02$) remained independently associated with RF and/or anti-CCP positivity. In men, a history of smoking (RF: OR 2.12, 95% CI 1.19-3.77, $P = 0.01$; anti-CCP: OR 1.87, 95% CI

Table. Clinical characteristics of patients at the time of diagnosis of RA in this study.

	All, N = 1685	RF Positive, n = 1294	RF Negative, n = 391	*P*	Anti-CCP Positive, n = 1262	Anti-CCP Negative, n = 423	*P*
Female sex	1406 (83.4)	1118 (86.4)	288 (73.7)	**< 0.001**	1097 (86.9)	309 (73)	**< 0.001**
Age at diagnosis, yrs, mean (SD)	51.9 (15.4)	50.8 (15.0)	55.9 (16.1)	**< 0.001**	50.2 (14.9)	56.9 (14.8)	**< 0.001**
< 30	169 (10)	136 (10.5)	33 (8.4)		135 (10.7)	34 (8)	
30-39	215 (12.8)	181 (14)	34 (8.7)		188 (14.9)	27 (6.4)	
40-49	322 (19.1)	261 (20.2)	61 (15.6)		263 (20.8)	59 (13.9)	
50-59	427 (25.3)	333 (25.7)	94 (24)		316 (25)	111 (26.2)	
60-69	346 (20.5)	258 (19.9)	88 (22.5)		244 (19.3)	102 (24.1)	
70-79	171 (10.1)	107 (8.3)	64 (16.4)		103 (8.2)	68 (16.1)	
≥ 80	35 (2.1)	18 (1.4)	17 (4.3)		13 (1)	22 (5.2)	
Smoking history[a]	370 (24.9)	294 (22.7)	76 (19.4)	0.23	288 (22.8)	82 (19.4)	0.17
Family history of RA[b]	142 (9.6)	119 (9.2)	23 (5.9)	**0.04**	110 (8.7)	32 (7.6)	0.54
BMI[c], kg/m^2, mean (SD)	21.6 (3.3)	21.5 (3.3)	21.9 (3.3)	0.05	21.5 (3.3)	22.0 (3.3)	**0.02**
BMI ≥ 25	197 (21.5)	140 (10.8)	57 (14.6)	0.06	136 (10.8)	61 (14.4)	**0.03**
Serology							
RF positive	1294 (76.8)	–	–	–	1167 (92.5)	127 (30.3)	**< 0.001**
Anti-CCP positive	1262 (74.9)	1167 (90.2)	95 (24.3)	**< 0.001**	–	–	–
Steinbrocker stage ≥ 2	1391 (82.6)	1086 (83.9)	305 (78)	**0.01**	1074 (85.1)	317 (74.9)	**< 0.001**
ILD	134 (8)	118 (9.1)	16 (4.1)	**< 0.001**	118 (9.4)	16 (3.8)	**< 0.001**

Values are n (%) unless indicated otherwise. Values in bold are statistically significant. [a] Smoking history, n = 1488. [b] Family history of RA, n = 1485. [c] BMI, n = 1384. Anti-CCP: anticyclic citrullinated peptide antibody; ILD: interstitial lung disease; RA: rheumatoid arthritis; RF: rheumatoid factor.

1.08-3.25, $P = 0.03$) and a family history of RA (RF: OR 4.91, 95% CI 1.08-22.20, $P < 0.001$) remained independently associated with RF and/or anti-CCP positivity. Age was no longer a significantly associated variable.

Analysis performed after subcategorization based on patients' smoking habits and BMI showed that sex remained significantly associated with both RF and anti-CCP positivity in each subgroup; however, age was significantly associated only in patients without a history of smoking (RF: OR 0.98, 95% CI 0.97-0.99, $P < 0.001$; anti-CCP: OR 0.97, 95% CI 0.96-0.98, $P < 0.001$) and in those with BMI < 25 (RF: OR 0.98, 95% CI 0.97-0.99, $P < 0.001$; anti-CCP: OR 0.97, 95% CI 0.97-0.98, $P < 0.001$). Multivariable analysis showed that BMI was not independently associated with RF or anti-CCP positivity in any subgroup.

Association between age at onset of RA and autoantibody formation. When patients were categorized based on age at the time of diagnosis in 10-year age brackets, RF and anti-CCP positivity significantly declined (RF: 80.5%, 84.2%, 81.1%, 78%, 74.6%, 62.6%, and 51.4%, $P < 0.001$; anti-CCP: 79.9% 87.4%, 81.7%, 74%, 70.5%, 60.2%, and 37.1%, $P < 0.001$; double positivity: 74.6%, 79.1%, 75.8%, 68.4%, 65%, 57.3%, and 34.3%, $P < 0.001$; Supplementary Figure S1 and S2, available with the online version of this article). Further analysis after subcategorization based on sex, smoking habit, and BMI ≥ 25 showed that seropositivity decreased significantly with age in women, in nonsmokers, and in patients with BMI < 25. This is in contrast with the disappearance of this association in men, in those with a history of smoking, and in those with BMI ≥ 25 (Figure 1 and Supplementary Table S1). RA serology was significantly associated with age regardless of a family history of RA.

Effect of aging on RA serology subcategorized based on a combination of sex, smoking habit, and family history. We further investigated the association between a combination of age, sex, smoking habit, and family history, which were independently associated with RA serology. Figure 2 shows the RF and anti-CCP positivity rates for each combination of age, sex, smoking habit, and family history. These findings indicate that RA serology may be affected not by a single variable but by a complex interplay between multiple factors.

DISCUSSION

We investigated factors associated with RA serology in this large-scale cohort of patients with RA and observed that age at disease onset, sex, smoking habit, and family history were independently associated with RF and anti-CCP positivity.

RF and anti-CCP, which represent autoantibodies specific for RA, were strongly associated with the pathophysiology of RA and are useful for clinical diagnosis and prediction of prognosis in patients with RA.[2,3] Autoantibody production is affected by various factors, which are broadly classified into genetic and environmental factors.[1] HLA-DRB1 shared epitope is the most common genetic factor, and cigarette smoke, periodontal disease, microbiota, and obesity are implicated as major environmental

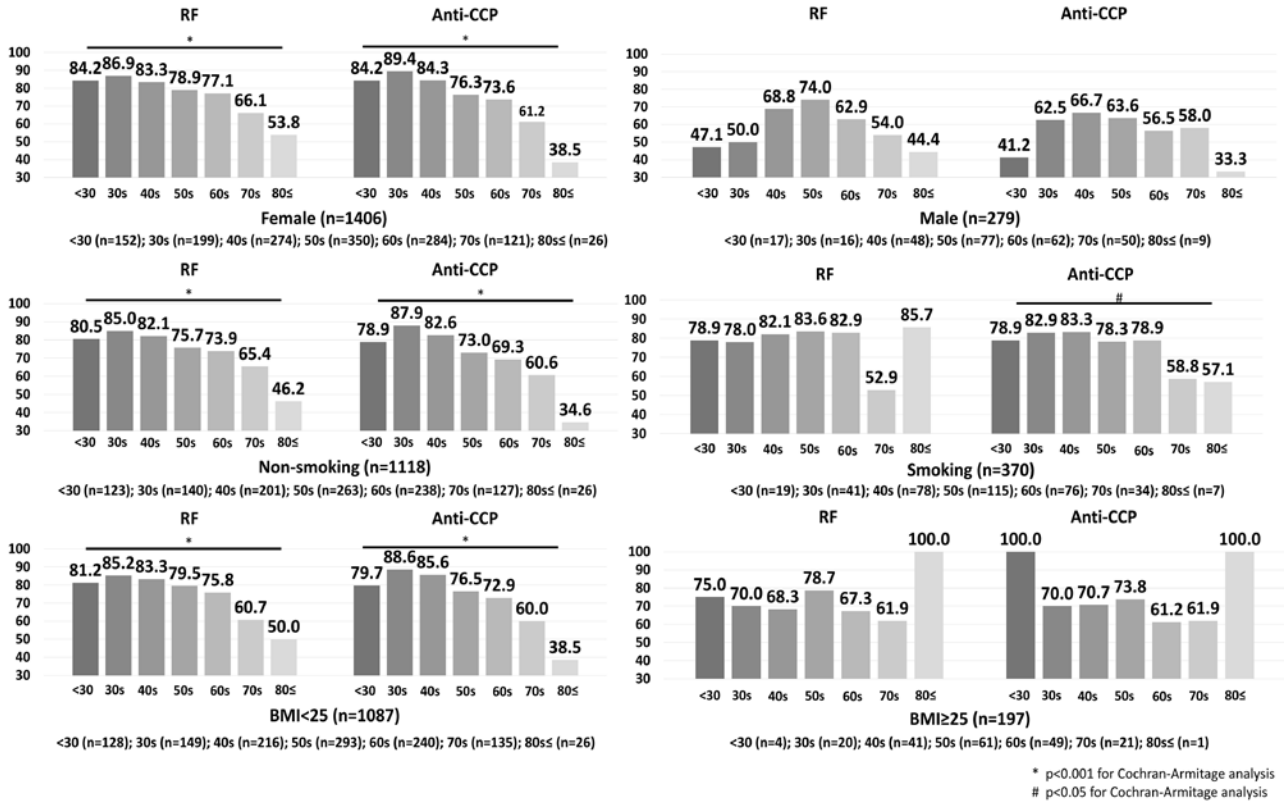

Figure 1. Trend of positivity of RF and anti-CCP subdivided by sex, smoking habit, and BMI. Tendency of decreasing seropositivity along with age were significant in patients who were female, had a nonsmoking history, and a BMI < 25. This tendency disappeared in patients who were male, had a smoking history, and a BMI ≥ 25. Anti-CCP: anticyclic citrullinated peptide antibody; RF: rheumatoid factor.

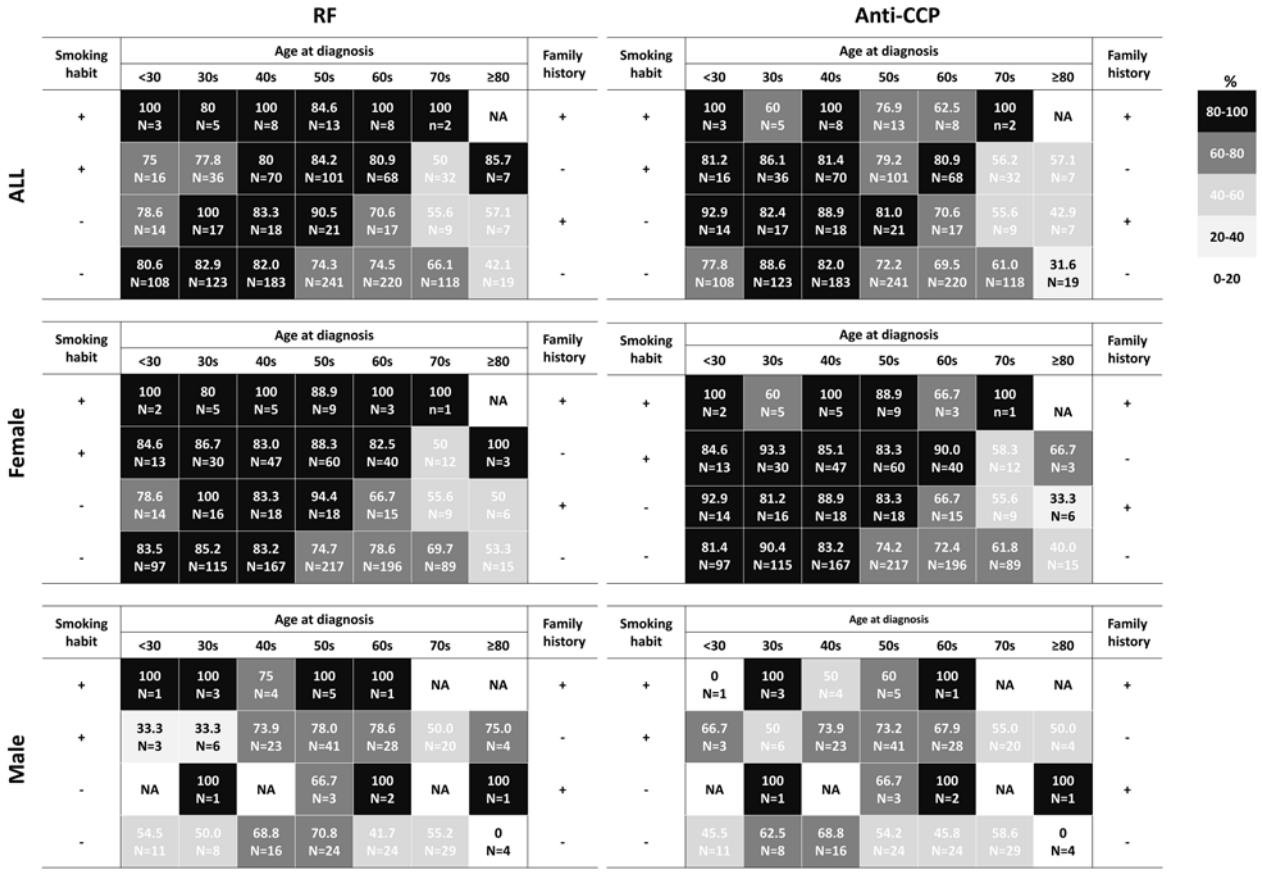

Figure 2. Effect of aging on serology of RA subdivided based on a combination of sex, smoking habit, and family history. Positivity of RF and anti-CCP are determined by the complex interplay of multiple relevant factors. Anti-CCP: anticyclic citrullinated peptide antibody; RA: rheumatoid arthritis; RF: rheumatoid factor.

factors that contribute to autoantibody production.[1] Our study also showed that age was independently associated with RA seronegativity. Aging is known to be associated with significant alterations in the immune system, which is referred to as immunosenescence, characterized by activation of the innate immune system and decreased T and B lymphocyte activity of acquired immunity.[11] Intrinsic B cell function declines with age, resulting in reduced antibody production and decreased affinity maturation on an antibody response, which may also be attributed to decreased autoantibody production.[12] The increase in RA seronegativity observed in our study may be attributable to immunosenescence. Aging is also associated with inflammageing, which refers to unresolved systemic inflammation in the absence of pathogens.[13] Significant inflammation observed in patients with elderly-onset RA corroborates this finding reported by previous studies.[6,7] In addition, recent single-cell sequencing of anti-CCP–positive and –negative patients with RA revealed differential involvement of cellular and molecular pathways in the pathogenesis.[14] These data indicate that seronegative and seropositive RA have different genetic pathogeneses, although their clinical phenotypes may be similar.

Our study showed a low prevalence of autoantibodies in men regardless of age, whereas age was significantly associated with seronegativity in women. RA occurred more frequently in women than in men across all age groups; however, sex-based differences were more pronounced in premenopausal women.[6,15] Interestingly, studies have reported that the risk of seronegative RA increases with rapid reduction in endogenous serum estrogen levels during the postpartum period.[16] Menopause is associated with significant alterations in sex hormone balance, similar to those observed during the postpartum period. A large national cohort study reported a significant association between the postmenopausal status and RA seronegativity.[17] Therefore, the increased prevalence of seronegativity in men and in elderly women observed in our study may be attributable to the association with sex hormones.

Smoking is a known risk factor for the development of autoantibodies. A recent study observed that the association between smoking and high anti-CCP levels was more prominent in cases of shared epitope alleles; however, high RF levels were shown to be independent of shared epitope alleles,[18] which highlights the synergistic effects of environmental and genetic factors in autoantibody production. Therefore, RA serology is affected by a complex interplay between many genetic and environmental factors, and their effects may differ based on age-induced physiological changes.

Recent studies have reported an increase in the age at onset of RA.[6,19] Elderly-onset RA is characterized by acute onset, seronegativity, a pattern of predominantly large joint involvement, and significant inflammation, all of which distinguish this

condition from young-onset RA.[6,7] Because polymyalgia rheumatica (PMR) or calcium pyrophosphate dehydrate deposition disease (CPPD) can share similar clinical features with RA, rheumatologists should pay attention to possible misdiagnosis.

In addition to clinical features, ultrasonography documented residual synovitis. Further, the histological synovitis score post-treatment and radiographic progression in elderly-onset RA were reportedly significantly poorer than observed in young-onset RA, which indicates that elderly patients may show treatment refractoriness.[20] An increase in the prevalence of seronegativity rates and a change in the disease pattern of RA are expected in the future as a result of rapid population aging. Our data call attention to the need for a treatment strategy for RA considering various patient profiles; notably, data reported by previous studies have not specifically focused on elderly patients.[21,22]

This study has limitations. First, the single-center design of this study performed in Japan may limit its generalizability. Second, anti-CCP screening was not performed at the time of diagnosis in some patients. However, in view of the rarity of anti-CCP seroconversion, this issue may not be a major drawback. Third, RF screening was performed at the time of diagnosis and not necessarily followed-up in clinical practice. Due to the nature of a retrospective study, we could not gather the information regarding seroconversion of RF. Fourth, we did not consider periodontal disease in this study because gathering accurate data from patients' clinical records was challenging. Fifth, this is a descriptive study without accompanying basic research data. Therefore, we cannot say the effect of immunosenescence definitively on the increase in seronegative RA without autoantibodies. However, our study warrants further basic studies to elucidate the relationship between immunosenescence and serology in autoimmune diseases. Finally, although we carefully diagnosed patients with negative autoantibodies as seronegative RA, there remains possibility of misdiagnosis or complications with PMR or CPPD, which share similar clinical features with RA. Large-scale prospective studies using multinational collaborative cohorts are warranted to validate our findings.

In conclusion, our study highlights that age was independently associated with seronegative RA and that the effect of age varied depending on the combination of age with other relevant variables. Further studies are required to determine the factors associated with seropositivity in patients with RA and to conclusively establish the optimal therapeutic approach for the management of RA.

DATA AVAILABILITY

The data underlying this article will be shared on reasonable request to the corresponding author.

ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

REFERENCES

1. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. Lancet 2016;388:2023-38.
2. Aletaha D, Neogi T, Silman AJ, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 2010;69:1580-8.
3. Kaneko Y, Kuwana M, Kameda H, Takeuchi T. Sensitivity and specificity of 2010 rheumatoid arthritis classification criteria. Rheumatology 2011;50:1268-74.
4. Myasoedova E, Davis J, Matteson EL, Crowson CS. Is the epidemiology of rheumatoid arthritis changing? Results from a population-based incidence study, 1985-2014. Ann Rheum Dis 2020;79:440-4.
5. Matthijssen XME, Huizinga TWJ, van der Helm-van Mil AHM. Increasing incidence of autoantibody-negative RA is replicated and is partly explained by an aging population. Ann Rheum Dis 2022;81:e69.
6. Uchiyama S, Takanashi S, Takeno M, et al. Should we reconsider the definition of elderly-onset rheumatoid arthritis in an ageing society? Mod Rheumatol 2022;32:323-9.
7. Deal CL, Meenan RF, Goldenberg DL, et al. The clinical features of elderly-onset rheumatoid arthritis. A comparison with younger-onset disease of similar duration. Arthritis Rheum 1985;28:987-94.
8. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
9. Steinbrocker O, Traeger CH, Batterman RC. Therapeutic criteria in rheumatoid arthritis. J Am Med Assoc 1949;140:659-62.
10. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 2013;48:452-8.
11. Mogilenko DA, Shchukina I, Artyomov MN. Immune ageing at single-cell resolution. Nat Rev Immunol 2022;22:484-98.
12. de Mol J, Kuiper J, Tsiantoulas D, Foks AC. The dynamics of B cell aging in health and disease. Front Immunol 2021;12:733566.
13. Morrisette-Thomas V, Cohen AA, Fülöp T, et al. Inflamm-aging does not simply reflect increases in pro-inflammatory markers. Mech Ageing Dev 2014;139:49-57.
14. Wu X, Liu Y, Jin S, et al. Single-cell sequencing of immune cells from anticitrullinated peptide antibody positive and negative rheumatoid arthritis. Nat Commun 2021;12:4977.
15. Kvien TK, Uhlig T, Ødegård S, Heiberg MS. Epidemiological aspects of rheumatoid arthritis: the sex ratio. Ann N Y Acad Sci 2006;1069:212-22.
16. Orellana C, Wedrén S, Källberg H, et al. Parity and the risk of developing rheumatoid arthritis: results from the Swedish Epidemiological Investigation of Rheumatoid Arthritis study. Ann Rheum Dis 2014;73:752-5.
17. Bengtsson C, Malspeis S, Orellana C, Sparks JA, Costenbader KH, Karlson EW. Association between menopausal factors and the risk of seronegative and seropositive rheumatoid arthritis: results from the Nurses' Health Studies. Arthritis Care Res 2017;69:1676-84.
18. Ishikawa Y, Ikari K, Hashimoto M, et al. Shared epitope defines distinct associations of cigarette smoking with levels of anticitrullinated protein antibody and rheumatoid factor. Ann Rheum Dis 2019;78:1480-7.
19. Kato E, Sawada T, Tahara K, et al. The age at onset of rheumatoid arthritis is increasing in Japan: a nationwide database study. Int J Rheum Dis 2017;20:839-45.
20. Romão VC, Humby F, Kelly S, et al. Treatment-resistant synovitis and radiographic progression are increased in elderly-onset rheumatoid arthritis patients: findings from a prospective observational longitudinal early arthritis cohort study. Semin Arthritis Rheum 2020;50:735-43.
21. Takanashi S, Kaneko Y, Takeuchi T. CDAI and DAS28 in the management of rheumatoid arthritis in clinical practice. Ann Rheum Dis 2020;79:671-4.
22. Takanashi S, Kaneko Y, Takeuchi T. Characteristics of patients with difficult-to-treat rheumatoid arthritis in clinical practice. Rheumatology 2021;60:5247-56.