

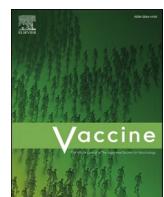


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Author(s)	Yamaguchi, Yuta; Amiya, Saori; Inokuchi, Shoichiro et al.
Citation	Vaccine. 2025, 66, p. 127771
Version Type	VoR
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Distinct trajectories of humoral immune responses after SARS-CoV-2 mRNA vaccination in autoimmune inflammatory rheumatic diseases: A group-based trajectory analysis

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ARTICLE INFO

Keywords:

Severe acute respiratory syndrome coronavirus 2
 Coronavirus disease 2019
 Multiple mRNA vaccination
 Group-based trajectory modelling

ABSTRACT

Background: Patients with autoimmune inflammatory rheumatic diseases (AIRDs) are at a high risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Data on the effects of multiple (>3) vaccine doses and their influencing factors are limited. Understanding antibody dynamics after repeated vaccinations is essential for optimising vaccination strategies. Therefore, this study characterised the antibody response trajectories against SARS-CoV-2 and explored key clinical and immunological determinants.

Methods: This single-centre retrospective cohort study included patients with AIRDs who received SARS-CoV-2 vaccinations between 1 February and 6 December 2021. Serum neutralising antibody titres from the first to fifth vaccinations were analysed using group-based trajectory modelling (GBTM). The clinical characteristics and serum cytokine levels were compared between the response groups.

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Results: The study included 293 patients with AIRDs. Three response trajectories were identified via GBTM: low responders ($n = 13$, 4.4%), middle responders ($n = 122$, 41.6%), and high responders ($n = 158$, 54.0%). Abatacept use was the strongest predictor of a low response (odds ratio 11.55, [95% confidence interval 2.27–58.82], $p = 0.0032$) but was also associated with a middle response (3.82, [1.18–12.29], $p = 0.025$). A middle response was also linked to older age (2.31 [1.38–3.85], $p = 0.0014$), mycophenolate mofetil use (3.03 [1.02–8.97], $p = 0.045$), anti-neutrophil cytoplasmic autoantibody-associated vasculitis (3.26 [1.29–8.19], $p = 0.012$), and rheumatoid arthritis (1.62 [1.02–8.97], $p = 0.048$), while systemic lupus erythematosus was inversely associated (0.49 [0.26–0.95], $p = 0.035$). Low responders exhibited elevated inflammatory mediators, including interleukin-6 and B-cell activating factor.

Conclusion: We identified distinct antibody response trajectories in patients with AIRDs, including a subgroup with persistently low humoral immune responses despite repeated vaccinations. This study highlights the need for personalised vaccination strategies that consider individual clinical and immunological factors to optimise protection in this vulnerable populations.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) poses a significant threat to patients with autoimmune inflammatory rheumatic diseases (AIRDs) [1–4]. Although mRNA vaccines are a key preventive strategy for patients with and without AIRDs [5,6], immunosuppressive therapies, including glucocorticoids [7,8], methotrexate (MTX) [9–11], tumor necrosis factor- α inhibitors (TNFi) [8], abatacept (ABT) [7–10], mycophenolate mofetil (MMF) [11], belimumab [10], and B-cell depleting agents [7–11], are known to attenuate vaccine-induced immunity.

Repeated booster vaccinations are recommended for immunosuppressed individuals [12]. Individuals who failed to achieve seroconversion after two doses have successfully responded to a third dose [13], and repeated boosters have been associated with reduced infection and disease severity [6,14–16]. However, the effects of immunosuppressive treatments on vaccine efficacy remain a concern [17–19]. Despite the evidence of short-term vaccine efficacy, the long-term trajectory of SARS-CoV-2 immunity in patients with AIRDs receiving multiple mRNA vaccines remains poorly understood.

A critical unanswered question is whether antibody response trajectories in patients with AIRDs can be clearly classified, and if so, how these trajectories correlate with specific clinical or immunological factors. Identifying these patterns and related factors could improve risk stratification and inform tailored vaccination strategies. Group-based trajectory modelling (GBTM) is a statistical approach that classifies individuals into subgroups with similar response trajectories based on repeated measurements [20–22]. This method provides a statistically valid grouping based on actual outcomes rather than an arbitrary classification, allowing for a comprehensive analysis of the factors that characterise a particular trajectory. For instance, distinct pulmonary artery pressure trajectories in systemic sclerosis have been identified using GBTM, enabling more precise risk stratification and personalised management strategies [23].

We hypothesised that patients with AIRDs exhibit distinct neutralising antibody (NAb) response trajectories shaped by their clinical and immunological profiles. Therefore, we used GBTM to characterise the long-term antibody dynamics over multiple vaccinations and identify the key factors influencing vaccine efficacy, aiming to improve patient-oriented vaccination strategies for this vulnerable population.

2. Material and methods

2.1. Study design and participants

This prospective longitudinal observational study of SARS-CoV-2 mRNA vaccines was conducted from 1 February 2021 to 30 August 2024. Patients diagnosed with AIRDs who had agreed to participate in our previous study [24] and visited the Department of Clinical Immunology at The University of Osaka Hospital were included in this study.

Participation was voluntary, and all participants provided written informed consent. The local ethics committee of The University of Osaka Hospital approved this study (IRB no. 20118).

Patients were administered a questionnaire about the coronavirus disease 2019 (COVID-19) vaccine during their outpatient visit and asked to complete and return the questionnaire after vaccination. The completed questionnaires were returned in a sealed envelope directly to a central facility where a data manager entered the data into The University of Osaka Hospital's electronic medical records. Data on diagnoses, comorbidities, treatments, and demographics (age, sex, history of SARS-CoV-2 infection, vaccination date, and vaccine type) were extracted from the electronic medical records. Since this study did not provide vaccines, the vaccination schedule, interval, and vaccine type (BNT162b2 or mRNA-1273) were at the patient's discretion. Individuals (1) who withdrew consent; (2) who did not return the questionnaire during the observation period; (3) with a history of SARS-CoV-2 infection before the first vaccination; (4) whose pre-vaccination antibody titre was above the cut-off value; and (5) whose samples were not collected at multiple time points were excluded.

2.2. Serum samples

As in our previous study [24], serum samples were obtained from patients without additional blood sampling by collecting all residual samples after outpatient clinical examinations. The samples were used to evaluate the dynamics of the antibody response after the first (Term1; 1–21 days) and second to fifth (Term2–5; 14–42 days) doses of the COVID-19 mRNA vaccination, depending on their availability. Serum samples were stored at -80°C until use. The same set of samples was used for SARS-CoV-2 NAb and cytokine measurements. To assess the effects of seasonal vaccination and breakthrough infections, samples for measuring antibody titres against the SARS-CoV-2 nucleocapsid protein and influenza virus-specific antigens were collected annually each spring (from 1 March to 31 May), depending on sample availability.

2.3. Serum SARS-CoV-2 NAb measurements

Serum NAb titres against wild-type SARS-CoV-2 were measured using an iFlash3000 (YHLO Biotech Co., Ltd., Shenzhen, China; YH-C6111) fully automated chemiluminescent immunoassay analyser and an iFlash-2019-nCoV NAb kit (YHLO Biotech Co., Ltd., Shenzhen, China; YH-C86109), as previously reported [24]. The results are expressed in arbitrary units/mL (AU/mL) of inhibitory activity, with a cut-off value of 10.0 AU/mL (≥ 10 AU/mL: positive, < 10 AU/mL: negative).

2.4. Latent class trajectory modelling

To identify the patterns of NAb changes in the vaccine dose over time, GBTM, which specialises in finite mixture modelling [20], was fitted to the NAb titre time series. An analysis was performed referencing

the proposed framework [20,25] and a previous report [23] to select the appropriate model [23]. The appropriate model was determined using the Bayesian information criterion (BIC). First, the number and shape of trajectories with the highest BIC and entropy values (>0.5) were selected. Next, models were screened based on the following adequacy criteria: (a) the average posterior probability of assignments for each trajectory was >0.7 , (b) the odds of correct classification for each trajectory were >5 , (c) the relative entropy was >0.5 , and (d) the minimum number of individuals assigned to each trajectory exceeded 4% of the total population. Of the models that met all the above criteria, one final model was selected based on the BIC and clinical interpretability of the number and shape of the trajectories. To assess the model's validity, we compared the antibody titre results against those of the influenza virus, measured as described below, for each identified trajectory. To investigate the clinical phenotypes characterising each identified trajectory, the clinical characteristics and serum cytokines of patients with AIRDs were examined. As candidate factors, 26 variables were pre-specified according to clinical perspectives: age (young <40 years; middle: 40–64 years; old ≥ 65 years), sex, diagnosis (rheumatoid arthritis [RA], polymyalgia rheumatica, systemic lupus erythematosus [SLE], Sjogren syndrome, myositis, mixed connective tissue disease, SSc, anti-neutrophil cytoplasmic autoantibody-associated vasculitis [AAV], large vessel vasculitis, Behcet's disease, IgG4 related disease, and other), and treatment (glucocorticoids, MTX, calcineurin inhibitors, MMF, azathioprine, TNFi, interleukin [IL]-6R inhibitors, ABT, Janus kinase inhibitors [JAKi], and belimumab). These baseline clinical factors were described for each trajectory, and their associations with each trajectory were evaluated.

Measurement of serum antibodies specific to SARS-CoV-2 and influenza virus antigens.

Serum levels of antibodies specific to the SARS-CoV-2 nucleocapsid protein and influenza virus antigens were quantified using a V-PLEX Respiratory Panel 4 (IgG) Kit (Meso Scale Discovery Inc., Rockville, MD, USA; K15707U), in accordance with the manufacturer's instructions.

2.5. Serum cytokine measurement

Serum cytokines obtained from vaccinated participants were quantified using the LEGENDplex Human B Cell Panel (BioLegend, San Diego, CA, USA; 740,527) and Human Inflammation Panel 1 (BioLegend, 740809) following the manufacturer's protocols. Samples were loaded onto a FACSCanto II flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). The obtained FCS files were analysed using the LEGENDplex Data Analysis Software Suite (BioLegend), an online cloud-based program (<https://legendplex.qognit.com/>).

2.6. Statistical analyses

Data are presented as medians and interquartile ranges (IQR) or means and standard deviations for continuous variables and frequencies and proportions for categorical variables. A common logarithmic (log10) transformation was applied to SARS-CoV-2 NAb and anti-influenza virus antibody titres, cytokine concentrations, and C-reactive protein levels as independent variables. Only donors with data available at two or more time points were included in the GBTM analysis. The magnitude of the association was described using point estimates of odds ratios and 95% confidence intervals. Since the Influenza A/Darwin strain was only included in the vaccine in the 2022/2023 and 2023/2024 seasons, the analysis focused on individuals who received vaccinations in both seasons. The analysis also focused on individuals who received the Influenza B/Phuket vaccine at least three times between the 2020/2021 and 2023/2024 seasons. Graphical representations and statistical analyses were performed using Stata (version 18.5; Stata Corp, College Station, Texas, USA) and R (version 4.4.1; R Development Core Team, Vienna, Austria) in rStudio (rStudio Corp., Boston, MA, USA), as well as JMP pro (version 17.1.0, SAS Institute Inc., Cary, NC, USA),

USA) and GraphPad Prism (version 10.2.2; GraphPad Software Inc., San Diego, CA, USA).

2.7. Role of the funding sources

The funders of this study were not involved in the study design, the collection, analysis, and interpretation of data, manuscript writing, or the decision to publish.

3. Results

3.1. Patient characteristics

The study enrolled 613 patients with AIRDs, and 293 were included in the final analysis (Fig. 1). The median age was 66.0 (IQR, 53.0 to 74.0), and 76.8% were female (Table 1). RA was the most frequently observed disease (43.0%), followed by SLE (17.7%) and AAV (8.2%). Treatments included glucocorticoids (54.3%; median dose: 4.0 mg/day [IQR: 3.0–6.0]), MTX (35.2%; median dose: 8.0 mg/week [IQR: 6.0–10.0]), and biological agents (33.4%; TNFi, IL-6 receptor inhibitors, ABT, or belimumab). Additionally, 9.2% of patients were not treated with immunosuppressive agents.

3.2. Group-based trajectory of SARS-CoV-2 NAb titres

The model selection criteria identified three trajectory groups (Fig. 2 and Supplementary Tables 1 and 2): low responders (13 patients, 4.4%), middle responders (122 patients, 41.6%), and high responders (158 patients, 53.9%). To determine how these groups responded to other vaccinations, we collected patient samples after the vaccination and epidemic seasons and followed the annual trends in antibody titres against Influenza A/Darwin and Influenza B/Phuket for each trajectory group identified using the SARS-CoV-2 NAb titre (Supplementary Fig. 1a). For the Influenza A/Darwin strain, which was introduced for vaccination during the observation period, antibody titres significantly differed among the groups, but these differences varied across years, which may have been influenced by the sample size. Similar to the SARS-CoV-2 vaccinations, high responders achieved higher antibody titres. In contrast, the antibody titres did not differ among the groups for the Influenza B/Phuket strain (Supplementary Fig. 1b). These findings suggest that the three trajectory groups identified based on the SARS-CoV-2 NAb responses also exhibit distinct patterns of immunogenicity against other vaccinations, highlighting the broader relevance of this grouping for vaccination strategies.

Additionally, some patients exhibited antibody responses indicative of breakthrough infections after mRNA vaccination, with positive anti-SARS-CoV-2 nucleocapsid protein antibody results observed in 11 of

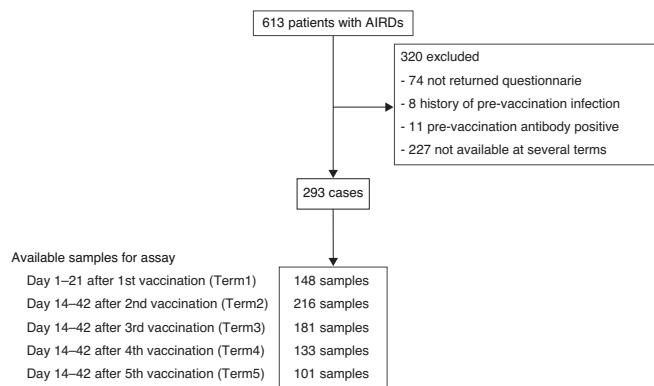


Fig. 1. Study population for the SARS-CoV-2 mRNA vaccination analysis. AIRD, autoimmune inflammatory rheumatic disease; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 1
Baseline clinical characteristics of the study population.

	All	High	Middle	Low
Age (years), median (IQR [range])	(n = 293) 66.0 (53.0–74.0)	(n = 158) 61.0 (49.0–71.0)	(n = 122) 70.0 (66.0–77.0)	(n = 13) 68.0 (48.5–75.5)
Female	225 (76.8)	125 (79.1)	90 (73.8)	10 (76.9)
AIRDs				
Rheumatoid arthritis	126 (43.0)	59 (37.3)	60 (49.2)	7 (53.8)
Systemic lupus erythematosus	52 (17.7)	35 (22.2)	15 (12.3)	2 (15.4)
ANCA-associated vasculitis	24 (8.2)	7 (4.4)	16 (13.1)	1 (7.7)
Large vessel vasculitis	10 (3.4)	6 (3.8)	4 (3.3)	0 (0.0)
Sjögren syndrome	9 (3.1)	8 (5.1)	1 (0.8)	0 (0.0)
Systemic sclerosis	14 (4.8)	9 (5.7)	5 (4.1)	0 (0.0)
Mixed connective tissue disease	8 (2.7)	6 (3.8)	2 (1.6)	0 (0.0)
Myositis	4 (1.4)	3 (1.9)	1 (0.8)	0 (0.0)
IgG4-related disease	8 (2.7)	6 (3.8)	2 (1.6)	0 (0.0)
Polymyalgia rheumatica	4 (1.4)	2 (1.3)	2 (1.6)	0 (0.0)
Behcet disease	5 (1.7)	2 (1.3)	3 (2.5)	0 (0.0)
Others [†]	29 (9.9)	15 (9.5) *	11 (9.0) **	3 (23.1) ***
Treatment for AIRDs				
Glucocorticoid	159 (54.3)	83 (52.5)	69 (56.6)	7 (53.8)
dose (mg/d), median (IQR)	4.0 (3.0–6.0)	4.0 (3.0–5.0)	5.0 (3.0–8.0)	5.0 (3.0–7.0)
Methotrexate	103 (35.2)	48 (30.4)	49 (40.2)	6 (46.2)
dose (mg/w), median (IQR)	8.0 (6.0–10.0)	8.0 (6.0–10.0)	6.0 (6.0–10.0)	8.0 (7.5–9.0)
Calcineurin inhibitors	41 (14.0)	23 (14.6)	15 (12.3)	3 (23.1)
Mycophenolate mofetil	17 (5.8)	5 (3.2)	11 (9.0)	1 (7.7)
Azathioprine	27 (9.2)	12 (7.6)	15 (12.3)	0 (0.0)
TNF- α inhibitors	27 (9.2)	12 (7.6)	14 (11.5)	1 (7.7)
IL-6R inhibitors	38 (13.0)	16 (10.1)	22 (18.0)	0 (0.0)
Abatacept	18 (6.1)	4 (2.5)	11 (9.0)	3 (23.1)
JAK inhibitors	11 (3.8)	5 (3.2)	4 (3.3)	2 (15.4)
Belimumab	15 (5.1)	8 (5.1)	5 (4.1)	2 (15.4)
without immunosuppressants	27 (9.2)	21 (13.3)	3 (2.5)	3 (23.1)

n (%) presented unless otherwise specified.

AIRD, autoimmune inflammatory rheumatic disease; ANCA, anti-neutrophil cytoplasmic antibody; IL-6, interleukin-6; JAK, Janus kinase; TNF, tumor necrosis factor.

[†] Acquired hemophilia (n = 1), adult-onset Still's disease (n = 4), anti-phospholipid antibody syndrome (n = 2), ankylosing spondylitis (n = 2), autoimmune hemolytic anemia (n = 1), common variable immunodeficiency (n = 3), hypereosinophilic syndrome (n = 2), Kimura disease (n = 1), mesenteric panniculitis (n = 1), polyarteritis nodosa (n = 1), psoriatic arthritis (n = 1), relapsing polychondritis (n = 2), sarcoidosis (n = 1), spondyloarthritis (n = 2), RS3PE syndrome (n = 1), SAPHO syndrome (n = 2), Castleman disease (n = 2).

* Adult-onset Still's disease (n = 2), anti-phospholipid antibody syndrome (n = 1), ankylosing spondylitis (n = 1), autoimmune hemolytic anemia (n = 1), hypereosinophilic syndrome (n = 1), Kimura disease (n = 1), mesenteric panniculitis (n = 1), psoriatic arthritis (n = 1), relapsing polychondritis (n = 1), sarcoidosis (n = 1), RS3PE syndrome (n = 1), SAPHO syndrome (n = 2), Castleman disease (n = 1).

** Acquired hemophilia (n = 1), Adult-onset Still's disease (n = 2), ankylosing spondylitis (n = 2), common variable immunodeficiency (n = 1), hypereosinophilic syndrome (n = 1), polyarteritis nodosa (n = 1), relapsing polychondritis (n = 1), spondyloarthritis (n = 1), Castleman disease (n = 1).

*** Anti-phospholipid antibody syndrome (n = 1), common variable immunodeficiency (n = 2).

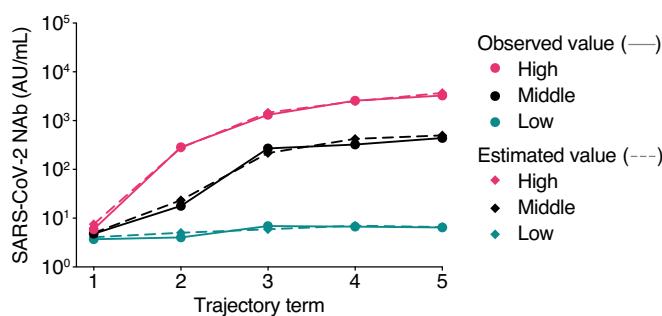


Fig. 2. Trajectories of NAbs against SARS-CoV-2 in patients with autoimmune inflammatory rheumatic diseases. Low, Middle, and High indicate low, middle, and high responders to the SARS-CoV-2 mRNA vaccination, respectively. Term1, 1–21 days after the first vaccination; Term2–5, 14–42 days after the second to fifth vaccinations, respectively.

NAb, neutralising antibody; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

225 patients in 2022, 38 of 225 patients in 2023, and 73 of 225 patients in 2024 (Supplementary Fig. 2a). Importantly, even after excluding these patients, the original GBTM-derived grouping pattern remained unchanged (Supplementary Fig. 2b). This consistency suggests that the group assignments derived from GBTM remain stable and that hybrid immunity resulting from vaccination and natural infections may have

limited influence on the observed grouping pattern.

3.3. Clinical characteristics of each trajectory group

Compared to the high-responder group, the low-responder group had a lower prevalence of SLE (15.4% vs 20.1%), a higher prevalence of RA (53.8% vs 39.6%), fewer patients who received IL-6 receptor inhibitors (0% vs 12.6%), and more patients who received ABT (23.1% vs 3.1%), JAKi (15.4% vs 3.1%), and belimumab (15.4% vs 5.0%). Furthermore, the middle-responder group had a lower prevalence of SLE (12.5% vs 20.1%), a higher prevalence of RA (48.2% vs 39.6%) and AAV (14.3% vs 4.4%), and more patients who received MTX (41.1% vs 32.1%) and MMF (9.8% vs 3.1%) (Table 1) compared to the high-responder group.

In the multinomial logistic regression using the high-responder group as the reference, ABT treatment was associated with the low- and middle-responder groups, with a stronger association observed in the low-responder group (Table 2). Older age, RA, AAV, and MMF were significantly associated with the middle-responder group (Table 2). Conversely, SLE was associated with a decreased likelihood of developing NAb titres compared with the reference group (Table 2).

3.4. Immunological characteristics of each trajectory group

A principal component (PC) analysis of 20 cytokines and four blood tests showed that the PC2 trend aligned more closely with the NAb titres in each trajectory group (Supplementary Fig. 3a and Supplementary

Table 2
Associations between patient characteristics and humoral immune response.

Baseline characteristics	Odds ratio (95% CI)		
	Low responder	Middle responder	High responder
sex			
Female	Reference	Reference	Reference
Male	1.14 (0.30, 4.37)	1.35 (0.77, 2.35)	Reference
Age			
24 to 39 years	NA	0.32 (0.09, 1.17)	Reference
40 to 64 years	Reference	Reference	Reference
65 to 87 years	1.17 (0.37, 3.65)	2.31 (1.38, 3.85)	Reference
AIRDs			
Rheumatoid arthritis	1.96 (0.63, 6.10)	1.62 (1.01, 2.62)	Reference
Systemic lupus erythematosus	0.64 (0.14, 3.02)	0.49 (0.26, 0.95)	Reference
ANCA-associated vasculitis	1.80 (0.20, 15.84)	3.26 (1.29, 8.19)	Reference
Large vessel vasculitis	NA	0.86 (0.24, 3.11)	Reference
Sjogren syndrome	NA	0.15 (0.02, 1.26)	Reference
Systemic sclerosis	NA	0.71 (0.23, 2.17)	Reference
Mixed connective tissue disease	NA	0.42 (0.08, 2.13)	Reference
Myositis	NA	0.43 (0.04, 4.16)	Reference
IgG4-related disease	NA	0.42 (0.08, 2.13)	Reference
Polymyalgia rheumatica	NA	1.30 (0.18, 9.36)	Reference
Behçet disease	NA	1.97 (0.32, 11.96)	Reference
Others [†]	2.86 (0.71, 11.55)	0.94 (0.42, 2.14)	Reference
Treatment for AIRDs			
Glucocorticoids	1.05 (0.34, 3.28)	1.18 (0.73, 1.89)	Reference
Methotrexate	1.96 (0.63, 6.15)	1.54 (0.94, 2.53)	Reference
Calcineurin inhibitors	1.76 (0.45, 6.88)	0.82 (0.41, 1.65)	Reference
Mycophenolate mofetil	2.55 (0.28, 23.62)	3.03 (1.02, 8.97)	Reference
Azathioprine	NA	1.71 (0.77, 3.79)	Reference
TNF- α inhibitors	1.01 (0.12, 8.47)	1.58 (0.70, 3.55)	Reference
IL-6R inhibitors	NA	1.95 (0.98, 3.91)	Reference
Abatacept	11.55 (2.27, 58.82)	3.82 (1.18, 12.29)	Reference
JAK inhibitors	5.56 (0.97, 32.02)	1.04 (0.27, 3.95)	Reference
Belimumab	3.41 (0.64, 18.04)	0.80 (0.26, 2.51)	Reference

AIRD, autoimmune inflammatory rheumatic disease; ANCA, anti-neutrophil cytoplasmic antibody; CI, confidence interval; IL-6R, interleukin-6 receptor; JAK, Janus kinase; TNF, tumor necrosis factor.

[†] Acquired hemophilia, adult-onset Still's disease, anti-phospholipid antibody syndrome, ankylosing spondylitis, autoimmune hemolytic anemia, common variable immunodeficiency, hypereosinophilic syndrome, Kimura disease, mesenteric panniculitis, polyarteritis nodosa, psoriatic arthritis, relapsing polychlorides, sarcoidosis, spondylarthritis, RS3PE syndrome, SAPHO syndrome, Castleman disease.

Tables 3–7). In Term1, some variables were only elevated in the low-responder group, excluding IL-17 A, monocyte chemoattractant protein 1 (i.e. MCP-1), BAFF, IL-8, and haemoglobin (Fig. 3a). BAFF and IL-8 levels decreased specifically in the high-responder group in Term1

compared to low- and middle-responder groups, whereas haemoglobin levels increased (Fig. 3a). In Term2, the BAFF and IL-6 levels were lowest in the high-responder group, followed by the middle- and low-responder groups (Fig. 3b). These factors were identified as major contributors in PC2 (Supplementary Fig. 3b). The haemoglobin levels showed the opposite trend in Term2 (Fig. 3b).

4. Discussion

This longitudinal cohort study evaluated patients with AIRDs who received multiple mRNA vaccinations against SARS-CoV-2 using GBTM for NAb titres from the first to fifth vaccinations. Our analysis identified three distinct response trajectories: (1) high responders who became seropositive after two doses and maintained high titres; (2) middle responders who achieved seropositivity after three doses; and (3) low responders who remained seronegative even after three doses. Notably, the trajectories did not intersect beyond the third dose. We further investigated the clinical and immunological features associated with each trajectory, revealing that age, disease type, immunosuppressive therapy, and inflammatory status significantly influenced the humoral immune response. These findings highlight the characteristics of patients whose adaptive immunity remains insufficient despite repeated vaccinations, emphasising the need for heightened vigilance regarding infection risk in these individuals.

Unlike conventional subgrouping based on disease type or treatment, GBTM enables data-driven classification based on antibody response dynamics rather than arbitrary criteria [20]. Although previous studies have used GBTM to assess SARS-CoV-2 antibody trajectories in healthy individuals [26], its application in patients with AIRDs remains limited. Our study provides novel evidence that patients with AIRDs exhibit distinct humoral response trajectories, with a particular focus on those who fail to mount a sufficient antibody response despite multiple boosters. Among the baseline clinical characteristics, ABT use was the strongest predictor of a poor antibody response. ABT inhibits the CD28-mediated co-stimulation of T cells via CD80/86 blockade, leading to impaired B cell activation and antibody production. A previous study related to mRNA vaccines highlighted IL-21, IL-2, and IFN- γ as key cytokines associated with impaired antibody responses induced by ABT [27]. However, our results appear to contradict these earlier findings. One possible explanation for this discrepancy is the methodological difference between studies: whereas previous research assessed cytokine production from peripheral blood mononuclear cells stimulated ex vivo, we measured circulating serum cytokine levels at least 1 day after vaccination. Further research incorporating longitudinal analyses of cytokine dynamics is warranted to clarify whether short- or long-term post-vaccination cytokine responses influence antibody production.

Given these results, it is crucial to consider the role of immunosuppressive drugs in the context of vaccination. The European Alliance of Associations for Rheumatology does not recommend discontinuing immunosuppressive drugs, except for rituximab, because of the risk of disease relapse [28]. In contrast, the American College of Rheumatology suggests interrupting certain medications for 1–2 weeks after vaccination, such as belimumab, most conventional (e.g., MMF, MTX) and targeted (e.g., JAKi) immunomodulatory therapies, and subcutaneous ABT. Intravenous ABT should be administered 1 week before vaccination [29]. They failed to reach consensus on whether cytokine inhibitors, such as TNF- α or IL-6 receptor blockers, should be interrupted. For rituximab, given the complexity of the dosing, it is recommended that clinicians and patients discuss the timing of vaccination individually. Although prospective studies are limited, one randomised controlled trial showed that interrupting MTX for 2 weeks after a booster vaccination increased antibody titre [30]. Another study found lower antibody titres in the ABT continuation group after booster vaccination, but the difference was not significant for TNFi [31]. Our study may provide further support for discontinuing ABT after booster vaccination.

To validate our classifications, we also analysed antibody responses

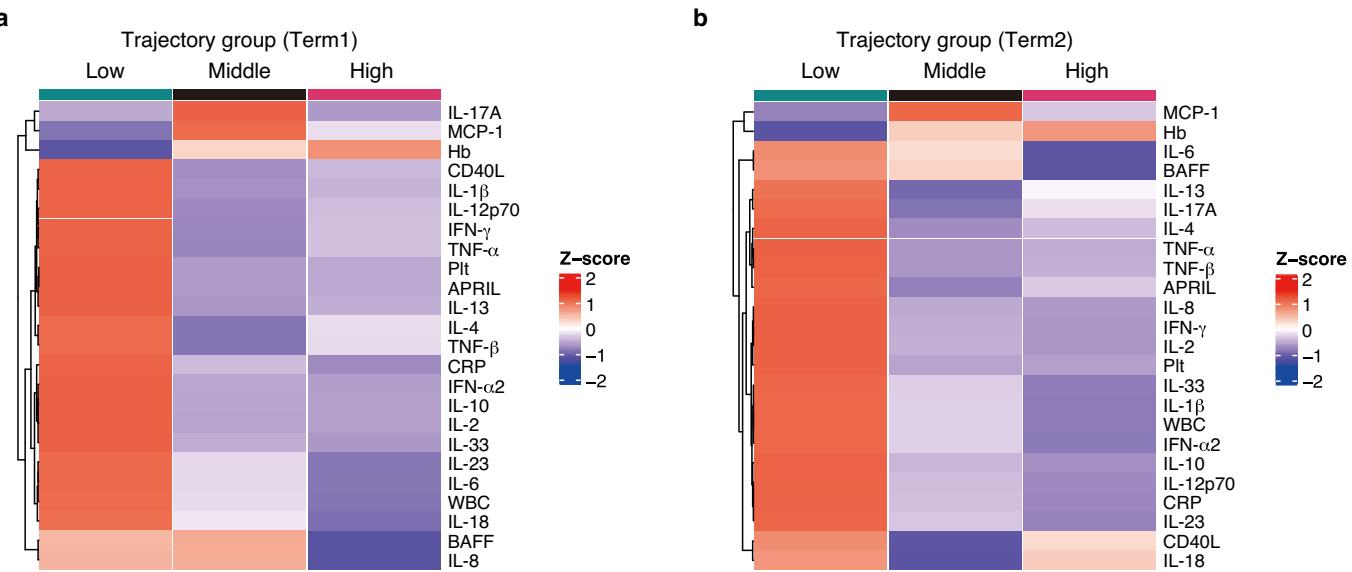


Fig. 3. Immunological characteristics of patients with autoimmune inflammatory rheumatic diseases in each trajectory identified using group-based trajectory modelling. (A, B) Heatmap of 20 cytokines, complete blood count (WBC, Hb, and Plt), and CRP at Term1 (A) and Term2 (B). Low, Middle, and High indicate low, middle, and high responders to the SARS-CoV-2 mRNA vaccination, respectively. Term1, 1–21 days after the first vaccination; Term2, 14–42 days after the second vaccination.

APRIL, A proliferation-inducing ligand; BAFF, B cell activating factor; CRP, C-reactive protein; Hb, haemoglobin; IFN, interferon; IL, interleukin; MCP, monocyte chemoattractant protein; Plt, platelet; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNF, tumor necrosis factor; WBC, white blood cells.

to influenza vaccines. The anti-Influenza A/Darwin antibody level was higher in the high-responder group than in the other groups. Although these findings primarily reflect humoral immunity rather than clinical outcomes, low haemagglutinin antibody titres correlate with an increased risk of influenza infection in healthy individuals [32], suggesting that low responders may also be at a higher risk of influenza infection [33]. However, our study did not investigate whether the low-responder group had higher influenza infection rates. Further studies incorporating cellular immune responses are warranted to better characterise protective immunity in this population.

RA and AAV were associated with a higher likelihood of belonging to the middle-responder group. Given that these diseases are driven by chronic inflammation, we examined cytokine profiles across response groups. In healthy individuals, reduced BAFF levels after influenza vaccination have been linked to increased antibody responses [34,35], which aligns with our findings for mRNA vaccination. However, previously reported positive associations with IL-10, CXCL13, BCMA, and APRIL were not consistently observed in our study. As our cohort consisted of patients with autoimmune diseases, cytokine levels may have been influenced by the underlying disease or immunosuppressive treatments, making direct comparisons with healthy populations challenging. Notably, we observed elevated levels of multiple cytokines in the poor-response group, suggesting that excessive systemic inflammation during the vaccination period might negatively affect vaccine efficacy.

This study had some limitations. First, this was a retrospective study; thus, the vaccination intervals, type of mRNA vaccine, and medication discontinuation before vaccination were not standardised. Second, this was a single-centre study conducted in Japan with a relatively small sample size, limiting our ability to independently analyse each disease and therapeutic agent. Future multicentre studies with larger cohorts are required to validate these findings. Third, NAb activity was evaluated using a surrogate virus neutralisation test (sVNT) because of the large number of serum samples. Although the sVNT correlates well with the gold-standard plaque reduction neutralisation test [36], it does not directly assess actual protective immunity [37]. Fourth, breakthrough infections were not systematically documented, potentially underestimating hybrid immunity. However, subgroup analysis excluding

patients with breakthrough infections supported our results (Supplementary Fig. 2a, b). Fifth, the significant diversity in autoimmune diseases and immunosuppressive treatments within our cohort may have contributed to heterogeneity in vaccine response patterns. Although our analysis primarily focused on overall response trajectories, additional subgroup analyses stratified by specific diagnoses and treatments would help to further elucidate individual-level influences on vaccine efficacy.

In conclusion, this study utilised GBTM to classify antibody response trajectories in patients with AIRDs and identified a subset that failed to achieve sufficient humoral immunity despite multiple booster vaccinations. Although this classification offers a valuable framework, larger cohorts and longitudinal follow-ups are essential to refine these groupings and fully elucidate their clinical significance. In the future, these insights may contribute to tailored vaccination strategies for patients with AIRDs.

CRediT authorship contribution statement

Yuta Yamaguchi: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Saori Amiya:** Writing – review & editing, Writing – original draft, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Shoichiro Inokuchi:** Writing – review & editing, Visualization, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Sayaka Nagao:** Resources, Investigation, Data curation. **Kazuma Kosaka:** Resources. **Shinichiro Nameki:** Resources, Data curation. **Teruaki Murakami:** Resources. **Yuko Yoshimine:** Resources. **Yasutaka Okita:** Resources. **Takahiro Kawasaki:** Resources. **Takayoshi Morita:** Resources. **Kohei Tsujimoto:** Resources. **Jun Fujimoto:** Resources. **Masayuki Nishida:** Resources. **Sumiyuki Nishida:** Resources. **Masashi Narazaki:** Resources. **Yasuhiro Kato:** Writing – review & editing, Writing – original draft, Resources, Investigation, Funding acquisition, Conceptualization. **Atsushi Kumanogoh:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Funding

This study was supported in part by research grants from Cloud Founding of Peace Winds Japan; the Center of Innovation program (COI STREAM) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT) (to A.K.); the Japan Society for the Promotion of Science (JSPS) KAKENHI (JP18H05282 to A.K., 22K16344 to Y.K.); the Japan Agency for Medical Research and Development (AMED) (JP223fa627002 to S.N. and A.K.); the Japan Agency for Medical Research and Development–Core Research for Evolutional Science and Technology (AMED–CREST) (J210705582, J200705023, J200705710, J200705049, JP18cm016335, JP18cm059042, 20kf0108454h0001, and 22gm1810003h0001 to A.K.); the Kansai Economic Federation (KANKEIREN) and Mitsubishi Zaidan (to A.K.). This work was also conducted as part of “The Nippon Foundation - The University of Osaka Project for Infectious Disease Prevention” and the All-Osaka U Research in “The Nippon Foundation - The University of Osaka Project for Infectious Disease Prevention”.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Atsushi Kumanogoh reports financial support was provided by Cloud Founding of Peace Winds Japan. Atsushi Kumanogoh reports financial support was provided by the Center of Innovation program (COI STREAM) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT) the Japan Society for the Promotion of Science (JSPS) KAKENHI. Atsushi Kumanogoh reports financial support was provided by the Japan Society for the Promotion of Science (JSPS) KAKENHI. Yasuhiro Kato reports financial support was provided by the Japan Society for the Promotion of Science (JSPS) KAKENHI. Atsushi Kumanogoh reports financial support was provided by the Japan Agency for Medical Research and Development (AMED). Atsushi Kumanogoh reports financial support was provided by the Kansai Economic Federation (KANKEIREN). Atsushi Kumanogoh reports financial support was provided by Mitsubishi Zaidan. This study was supported in part by research grants from the Japan Agency for Medical Research and Development–Core Research for Evolutional Science and Technology (AMED–CREST) to A.K. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to thank the patients and their families who participated in this study and the members of The University of Osaka Hospital for their help with collecting blood samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2025.127771>.

Data availability

Data will be made available on request.

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