Prediction of the intolerance or non-responder to Janus kinase inhibitors in patients with rheumatoid arthritis: a preliminary retrospective study with integrative cluster analysis

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Abstract

Objective

To identify the subpopulation of rheumatoid arthritis (RA) non-responders to Janus kinase inhibitors (JAKis) using cluster analysis.

Methods

This retrospective study enrolled RA patients who had been treated with JAKis (tofacitinib or baricitinib) between July 2013 and September 2019 in six centres. The endpoint was set as inadequate response to JAKis (JAKis-IR), defined as either non-response to JAKis or their intolerance. Non-response to JAKis was defined as achieving neither American College of Rheumatology 20% response nor Disease Activity Score (ΔDAS28-CRP) >1.2 at 12 weeks. Withdrawal time point included earlier than after 12 weeks from baseline. A hierarchical cluster analysis was performed with variables related with clinical and serological parameters at baseline.

Results

The 132 RA patients enrolled were classified into four groups (Group A-D). Groups consisted of three components defined at baseline, as seropositivity, advanced joint destruction, interstitial lung disease presumably associated with RA (RA-ILD). Group A (n=32): seronegative, presence of advanced joint destruction, absence of RA-ILD. Group B (n=35): seropositive, absence of advanced joint destruction and RA-ILD. Group C (n=20): seropositive, absence of advanced joint destruction, presence of RA-ILD. Group D (n=45): seropositive, presence of advanced joint destruction and RA-ILD. The rate of JAKis-IR in four groups was as follows: A, 34.3%; B, 17.1%; C, 20.0%; and D, 8.9%. The difference in JAKis-IR rate between group A and D was statistically significant.

Conclusion

A subpopulation of RA patients with a combination of the following three components, seronegativity, advanced joint destruction and absence of RA-ILD, was identified as being prone to JAKis-IR.

Key words

baricitinib, cluster analysis, rheumatoid arthritis, tofacitinib

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Introduction

Rheumatoid arthritis (RA) is characterised by chronic inflammation of multiple joints, which typically causes disability in the daily life of patients (1). RA is a heterogenous disease with respect to autoimmunity and clinical manifestations, including arthritis site, degree of progression of joint destruction, and complication of interstitial lung disease.

Janus kinase (JAK) inhibitors (JAKis) are competitive inhibitor that interact to adenosine triphosphate (ATP) binding site in the catalytic cleft of JAK kinase domain (2). JAK/signal transducers and activators of transcription (STAT) pathways are critical for immune cell activation, proinflammatory cytokine production, and cytokine signalling (3), being strongly responsible for the progression of RA (2-4). Many studies have indicated that JAKis are very potent to treat active RA patients (5, 6). Evidence for JAKis against RA, including their effect in RA patients with inadequate response to methotrexate (MTX), that in those with inadequate response to biological disease-modifying anti-rheumatic drugs (bDMARDs) and with MTX-naïve, has nowadays convinced rheumatologists their usefulness in daily clinical practice, and updated European League against Rheumatism (EULAR) recommendations for RA allowed a wider role for JAKis in the RA management strategy (7). However, there are still some patients who do not show enough response to JAKis treatment. Since previous high-quality phase III clinical trials focused on clarifying the efficacy and safety of JAKis in RA patients, there has been little information regarding the status of inadequate response to JAKis (JAKis-IR) in clinical practice (6, 8). In fact, high heterogeneity in real-world RA patients would make it more complex to consider what are the predictive factors for JAKis response.

Cluster analysis is a statistical method that identifies subgroups defined by a combination of factors and provides an unbiased categorisation of the subjects, allowing comparison of treatment responses between program-dependent subgroups. Cluster analysis has recently been applied to identify clinical and laboratory features in patients with some autoimmune diseases (9), and to evaluate prognosis (10).

In this preliminary study, we aimed to explore the subpopulation of JAKis-IR in patients with RA based on cluster analysis.

Methods

Patients and methods

We conducted a multicentre, retrospective pilot study at six centres. This research complied with the Declaration of Helsinki, and was approved by the local ethical review committee of Hokkaido University Hospital (approval no.: 017-0350) and by the ethics review board at each facility. Medical records were carefully reviewed retrospectively. Informed consent was given to all patients. Inclusion criteria were as follows: 1) Patients were diagnosed with RA who fulfilled the 1987 American College of Rheumatology (ACR) criteria (11) or the 2010 ACR/EULAR classification criteria for RA (1) . 2) Tofacitinib (Tofa) or baricitinib (Bari) were initiated during the time period July 2013 to September 2019 at standard dose (Tofa 10mg per day, Bari 4mg per day). 3) Patients were over 20 years of age. Exclusion criteria were as follows: 1) Patients refused to give informed consent, 2) Patients whose medical records did not have enough information to include this study, 3) Patients suspended JAKis due to reasons unrelated to the progression of RA, complications or infections, for example financial background. We performed a sample size calculation prior to study to estimate the size of patients enough to draw conclusions. We set confidence level:95%, margin of error: 5%, and population proportion:10%, and the estimated sample size was 139. The population proportion was set by referring to previous studies which assessed the efficiency of tofacitinib or baricitinib at 12 weeks, or long-term observation (12, 13) .

Cluster analysis

We applied hierarchical cluster analysis to aggregate RA patients into different groups sharing common characteristics according to the following variables at

the baseline: sex, age, disease duration, advanced joint destruction, global functional classification (Class III), interstitial lung disease presumably associated with RA (RA-ILD), complication with other autoimmune diseases (AIDs) including Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), dermatomyositis (DM), polymyositis (PM), systemic sclerosis (SSc), mixed connective tissue disease (MCTD), and vasculitis, anti-citrullinated protein antibody (ACPA), rheumatoid factor (RF), use/dose of MTX and prednisolone (PSL), erythrocyte sedimentation rate/C-reactive protein (ESR/CRP), tender/swollen joint counts (TJC/SJC), patient global assessment (PGA), and history of the use of bDMARDs. We determined variables for cluster analysis referred to a previous study from multiple point of view, namely epidemiological profiles, joint destruction and physical dysfunction, concomitant drugs, and activity of RA (13). Seropositive RA was defined as the presence of RF and/or ACPA, and RA patients negative for both RF and ACPA were considered seronegative. Advanced joint destruction was defined that more than one joint was identified with significant bone erosion, joint space narrowing, deformity, or ankylosis by x-ray, magnetic resonance imaging, and echography at baseline (14). We analysed shoulder, elbow, wrist, finger, hip, knee, ankle and toe joints, plus carpal and tarsus bones to detect advanced joint destruction. Global functional classification of RA was as follows: class I) completely able to perform usual activities of daily living, class II) able to perform usual self-care and vocational activities, to perform vocational and avocational activities, class IV) limited in ability to perform self-care, vocational, and avocational activities (15). RA-ILD was defined as interstitial lung lesion like ground-grass opacity, honeycombing and traction bronchiectasis evaluated by high-resolution computed tomography at baseline.

Euclidean distance and the Ward's agglomerative method were applied. Each variable was considered as a single cluster and combined with a neighbouring variable determined by the Euclidean distance. A dendrogram showed the process of clustering and the distance between clusters. To identify the ideal number of clusters, we decided to use clusters with reference to the dendrogram.

Table I. Background characteristics of RA patients treated with tofacitinib (Tofa) or baricitinib (Bari).

Factors	Total (n=132)	Tofa (n=67)	Bari (n=65)
Sex: female (n, %)	95 (72%)	47 (70%)	48 (74%)
Age (years old) (median, IQR)	60 (50-69)	58 (48-58)	63 (53-62)
Disease duration (month) (median, IQR)	93 (38-186)	89 (45-154)	109 (29-206)
Advanced joint destruction (n, %)	47 (36%)	26 (39%)	21 (32%)
ACR Class III (n, %)	19 (14%)	11 (16%)	8 (12%)
RF positive (n, %)	100 (76%)	56 (84%)	44 (68%)
RF titre (median, IQR)	74 (15-272)	85 (18-303)	69 (7-223)
ACPA positive (n, %)	102 (77%)	57 (85%)	45 (69%)
ACPA titre (median, IQR)	85 (6-300)	100 (24-300)	45 (1-293)
MTX use (median, %)	90 (68%)	43 (64%)	47 (70%)
MTX dose (mg/week) (median, IQR)	8 (0-10)	6 (0-10)	8 (0-12)
PSL use (median, %)	81 (61%)	42 (63%)	39 (58%)
PSL dose (mg/day) (median, IQR)	3 (0-5)	4 (0-6)	3 (0-5)
No history of bDMARDs (n, %)	32 (24%)	16 (24%)	16 (25%)
AIDs (n, %)	23 (17%)	12 (18%)	11 (17%)
RA-ILD (n, %)	33 (25%)	18 (27%)	15 (23%)
ESR (mm/1hour) (median, IQR)	26 (11-57)	35 (11-60)	21 (11-49)
CRP (mg/dL) (median, IQR)	0.44 (0.05-2.1)	0.75 (0.1-3.3)	0.22 (0.03-1.3)
TJC (median, IQR)	3 (2-6)	4 (2-7)	2 (2-6)
SJC (median, IQR)	3 (2-6)	4 (2-8)	3 (1-6)
PGA (median, IQR)	57 (37-71)	57 (37-76)	55 (37-70)
DAS28-CRP (median, IQR)	4.1 (3.1-4.9)	4.3 (3.5-4.9)	3.6 (2.8-4.6)

ACPA: Anti-citrullinated protein antibody; ACR: American College of Rheumatology; AIDs: Autoimmune diseases; bDMARDs: biological disease-modifying anti-rheumatic drugs; CRP: C-reactive protein; DAS28: Disease activity score; ESR: erythrocyte sedimentation rate; IQR: Interquartile range; MTX: Methotrexate; PSL: Prednisolone; PGA: Patient global assessment; RA: Rheumatoid arthritis; RA-ILD: Interstitial lung disease presumably associated with RA; RF: Rheumatoid factor; SJC: Swollen joint counts; TJC: Tender joint counts.

Endpoints

Disease activity was assessed using the Disease Activity Score (DAS28) and the ACR response criteria at 12 weeks after the initiation of JAKis treatment.

The endpoint was set as JAKis-IR, defined as either non-response to JAKis or their intolerance. In this study, JAKis non-response was defined as achieving neither ACR20 response nor ΔDAS28-CRP>1.2 at 12 weeks (16). Intolerance was defined as withdrawal of JAKis earlier than after 12 weeks from baseline due to exacerbation of arthritis, ingravescence of organ lesion related to RA, or adverse events. DAS28-CRP was calculated as follows (CRP: mg/dL): DAS28-CRP = $0.56*\sqrt{(TJC)} + 0.28*\sqrt{(SJC)} + 0.36*\log((CRP)*10+1) + 0.014*(PGA) + 0.96$ (16) . ACR20 was defined as follows: 1) over 20% improvement in TJC and SJC, 2) over 20% improvement in 3 of the following 5 areas: patient's pain visual analogue scale, patient's global visual analogue scale, physician's global visual analogue scale, patient's physical activity assessment, ESR or CRP (17).

Statistical analysis

Statistics for categorical variables were described as count and percentage. Continuous variables (age, disease duration, RF and ACPA at baseline, dose of MTX and PSL, ESR/CRP, tender/swollen joint counts, PGA) were expressed as median and quartiles. Non-parametric tests were conducted for comparisons. Fisher's exact test was applied for qualitative data analysis and Kruskal-Wallis test for quantitative data analysis. Ryan's procedure was used for multiple comparison of JAKis-IR rates according to cluster analysis. In all statistical analyses, p-value <0.05 was considered statistically significant. All statistical analyses were performed using JMP® Pro 14.2.0 (SAS Institute Inc., Cary, North Carolina, USA).

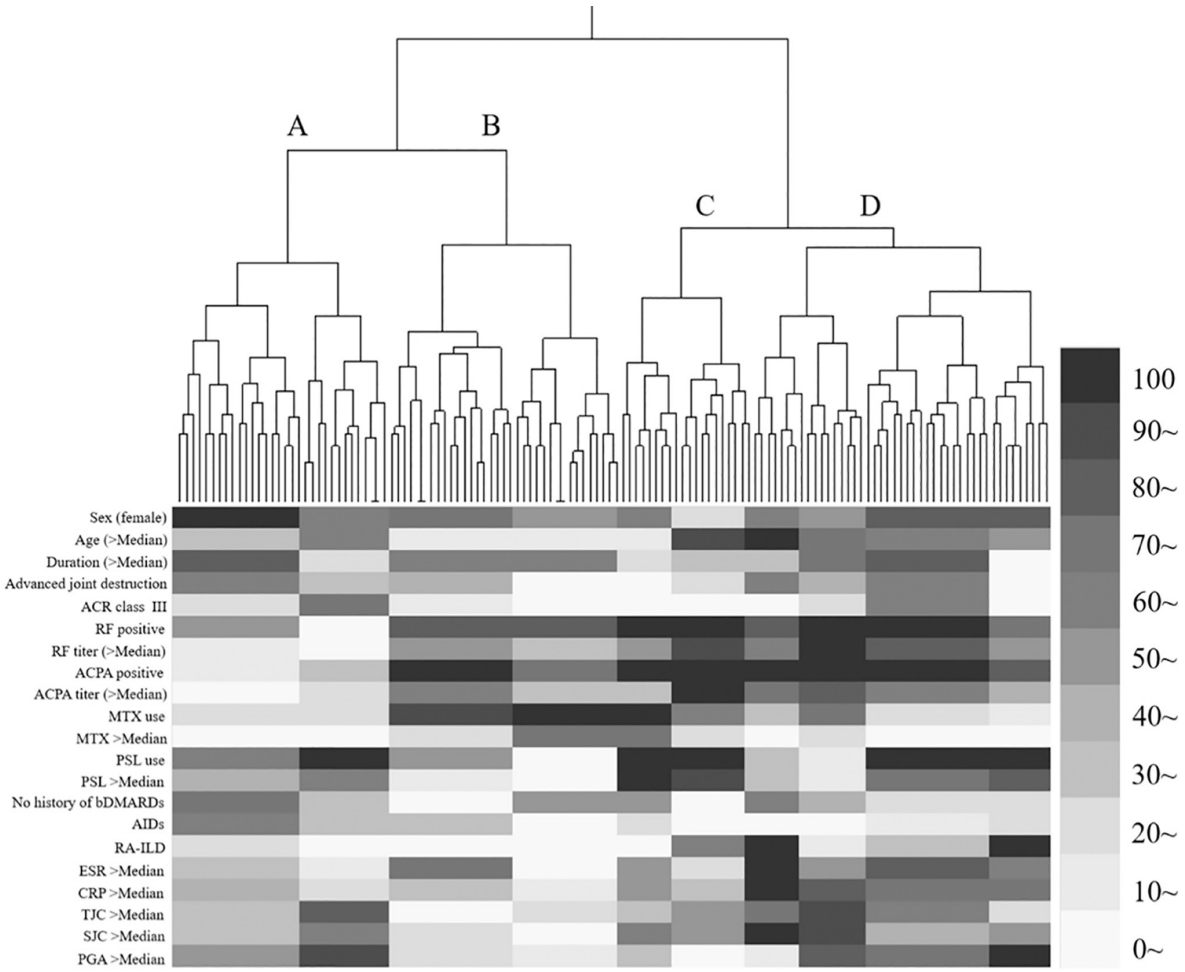


Fig. 1. Hierarchical cluster analysis and positive rate of each factor in subgroups.
Hierarchical cluster analysis classified the 132 RA patients into 4 clusters (**A**-**D**). The characteristics of 4 groups were as follows; Group A (n=32): seronegative, presence of advanced joint destruction but absence of RA-ILD, Group B (n=35): seropositive, absence of advanced joint destruction and RA-ILD. Group C (n=20): seropositive, absence of advanced joint destruction but presence of RA-ILD. Group D (n=45): seropositive, presence of advanced joint destruction and RA-ILD.

Results

A total of 171 consecutive patients with RA on JAKis were submitted from the 6 centres. Of them, 132 patients were enrolled in this study and 39 patients were excluded because they did not fulfil the inclusion criteria. Among enrolled patients, 67 patients were treated with Tofa, and 65 with Bari (Table I). We identified the four groups (A-D) with reference to the dendrogram based on the baseline data (Table I, Fig. 1). The groups were consisted of three components including seropositivity, advanced joint destruction, and presence of RA-ILD at baseline.

Group A: seronegative RA, presence of advanced joint destruction,and absence of RA-ILD

Group A comprised 32 patients (24.2% of the total cohort). The rate of seronegative RA was the highest in all 4 groups (22 patients, 69%) and the rate of advanced joint destruction (14 patients, 44%) was relatively high compared to group B (8 patients, 23%) and C (3 patients, 15%). The rate of RA-ILD was low compared to group C (7 patients, 35%) and D (24 patients, 53%).

Group B: seropositive RA, absence of advanced joint destruction, and absence of RA-ILD

Group B comprised 35 patients (26.5% of the total cohort). Most of the patients were seropositive RA (29 patients, 83%). The rate of advanced joint destruction was the second lowest in all groups and no patients had RA-ILD. The factors related to disease activity (ESR/CRP, TJC, SJC, PGA) were relatively lower than those of other groups. The rate of MTX use was the highest among the 4 groups (34 patients, 97%).

Group C: seropositive RA, absence of advanced joint destruction, and presence of RA-ILD

Group C comprised 20 patients (15.1% of the total cohort). All patients in Group C were seropositive and the rate of RA-ILD (7 patients, 35%) were higher than that of Group A and B. Male patients were more frequent than in other groups (12 patients, 60%). Sixteen patients (80%) used MTX and all patients in this group had PSL.

Group D: seropositive RA, presence of advanced joint destruction, and presence of RA-ILD

Group D comprised 45 patients (34.1%

Table II. Characteristics of RA patients in the 4 cluster groups.

Factors	A (n=32)	B (n=35)	C (n=20)	D (n=45)	*p*-value
Tofacitinib/Baricitinib (n)	12/20	22/13	9/11	24/21	0.20
Sex: female	28 (88%)	24 (69%)	8 (40%)	35 (78%)	0.002
Age (years old, median, IQR)	60 (50-68)	51 (47-58)	64 (51-67)	68 (59-74)	< 0.0001
Duration (month, median, IQR)	138 (49-242)	69 (48-149)	67 (18-117)	114 (39-233)	0.05
Advanced joint destruction (n, %)	14 (44%)	8 (23%)	3 (15%)	22 (49%)	0.01
ACR Class III (n, %)	8 (25%)	3 (9%)	1 (5%)	7 (16%)	0.15
RF positive (n, %)	8 (25%)	30 (86%)	20 (100%)	42 (93%)	<0.0001
RF titre (median, IQR)	6 (0-13)	68 (20-199)	155 (84-289)	239 (81-441)	<0.0001
ACPA positive (n, %)	7 (22%)	31 (89%)	20 (100%)	44 (98%)	<0.0001
ACPA titre (median, IQR)	0 (0-3)	88 (16-216)	260 (93-518)	219 (45-411)	<0.0001
Seropositive (n, %)	5 (16%)	29 (83%)	20 (100%)	41 (91%)	<0.0001
Seronegative (n, %)	22 (69%)	3 (9%)	0 (0%)	0 (0%)	<0.0001
MTX use (n, %)	26 (81%)	34 (97%)	16 (80%)	14 (31%)	<0.0001
MTX dose (median, range)	10 (8-12)	8 (6-12)	9 (6-12)	0 (0-4)	<0.0001
PSL use (n, %)	20 (63%)	10 (29%)	20 (100%)	31 (69%)	<0.0001
PSL dose (median, IQR)	4 (0-5)	0 (0-2)	5 (5-8)	5 (0-6)	<0.0001
No history of bDMARDs use (n, %)	4 (13%)	9 (26%)	4 (20%)	15 (33%)	0.20
History of TNF inhibitors use (n, %)	22 (69%)	22 (63%)	13 (65%)	21 (47%)	0.20
History of IL-6 inhibitors use (n, %)	16 (50%)	12 (34%)	11 (55%)	19 (42%)	0.42
History of abatacept use (n, %)	12 (38%)	10 (29%)	2 (10%)	20 (44%)	0.04
AIDs (n, %)	10 (31%)	7 (20%)	2 (10%)	4 (9%)	0.06
SS, SLE, DM/PM, SSc, MCTD, Others (n)	4/5/0/1/0/1	5/1/0/0/0/1	1/0/0/0/1/0	1/1/2/1/0/1	
RA-ILD (n, %)	2 (6%)	0 (0%)	7 (35%)	24 (53%)	<0.0001
ESR (median, IQR)	14 (9-30)	22 (8-43)	23 (5-49)	54 (21-92)	<0.0001
CRP (median, IQR)	0.11 (0.02-1.0)	0.1 (0.02-0.45)	0.29 (0.02-1.68)	1.56 (0.75-4)	<0.0001
TJC (median, IQR)	4 (2-9)	1 (1-2)	3 (2-6)	5 (2-9)	<0.0001
SJC (median, IQR)	3 (1-8)	2 (1-3)	5 (2-7)	5 (3-8)	<0.0001
PGA (median, IQR)	69 (54-80)	37 (23-50)	37 (28-48)	70 (57-80)	<0.0001
DAS28-CRP (median, IQR)	4.2 (3.3-4.8)	2.8 (3.3-3.6)	3.9 (3.2-4.4)	4.8 (4.2-5.3)	<0.0001

Binary values are number (percent) unless otherwise indicated.
ACPA: Anti-citrullinated protein antibody; ACR: American College of Rheumatology; AIDs: autoimmune diseases; bDMARDs: biological disease-modifying anti-rheumatic drugs; CRP: C-reactive protein; DAS28: Disease Activity Score; DM: dermatomyositis; ESR: erythrocyte sedimentation rate; IL-6: interleukin-6; IQR: interquartile range; MCTD: mixed connective tissue disease; MTX: methotrexate; PGA: patient global assessment; PM: polymyositis; PSL: prednisolone; RA: rheumatoid arthritis; RA-ILD: interstitial lung disease presumably associated with RA; RF: rheumatoid factor; Seronegative: RF and ACPA negative; Seropositive: RF and ACPA positive; SLE: Systemic lupus erythematosus; SJC: swollen joint counts; SS: Sjögren's syndrome; SSc: scleroderma; TJC: tender joint counts; TNF: tumour necrosis factor.
p-values <0.05 were considered statistically significant. *p*-values were calculated using Kruskal-Wallis test or Fisher's exact test and *p*-values <0.05 were considered statistically significant.

of the total cohort). Ninety-one percent (41 patients) of the patients were seropositive, and the rates of advanced joint destruction and RA-ILD were the highest in the 4 groups (49% and 53%, respectively). The factors related to disease activity (ESR, CRP, TJC, SJC, PGA) were higher in this group than in any other clusters. The rate of MTX use was the lowest in all groups (14 patients, 31%).

Comparison of JAKis-IR in the four groups

Of the 132 enrolled patients, 25 patients were identified as JAKis-IR. The number of patients who were not achieving ACR20, ΔDAS28-CRP<1.2 was 22 and 21, respectively (20 patients met both criteria). The number of patients who were intolerance of JAKis was 5: Group A: n=1, Group B: n=1, Group C: n=1, Group D: n=2. The rate of JAKis-IR in each cluster is shown in Figure 2. The significant difference in of JAKis-IR frequency was identified in 4 groups by Fisher's exact test (p=0.04), and between group A and D by Ryan's procedure. Sensitivity analysis including only patients completing the full 12 weeks period resulted in similar findings (Supplementary Fig. S1). Comparing the rate of JAKis-IR in 4 cluster groups (A-D) in this condition, significant difference in 4 groups was also identified by Fisher's exact test (p=0.01), and between group A and D by Ryan's procedure.

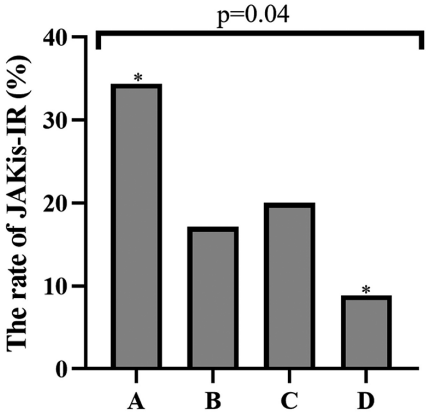
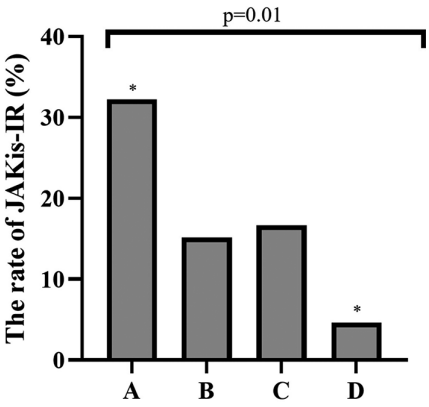


Fig. 2. The comparison of the rate of JAKis-IR in all patients in 4 cluster groups. Comparing the rate of JAKis-IR in 4 cluster groups (**A**-**D**), significant difference in 4 groups was identified by Fisher's exact test (p=0.04), and between group A and D by Ryan's procedure.

Discussion

In this study, we identified a subpopulation with JAKis-IR in patients with RA by cluster analysis based on three components including seropositivity, advanced joint destruction, and RA-ILD. A subpopulation with JAKis-IR (Group A) comprised the following



Supplementary Fig. S1.

characteristics: seronegative, presence of advanced joint destruction, absence of RA-ILD. The combination of cluster analysis and cluster-associated outcomes can be useful for the identification subgroups and the management of heterogenous diseases such as RA.

There has been little information regarding the predictive factors for JAKis response, except for seropositivity. In a *post-hoc* analysis of five Phase III studies of Tofa in patients with RA, seropositive patients were more likely to achieve ACR20/50/70 than seronegative patients who received Tofa 10mg at 3 months (18). JAKis-IR rate in the study was 30% in seropositive patients and 50% in seronegative patients, respectively. Our data in a univariate analysis showed a similar trend (seropositive 16%, seronegative 44%, data not shown). Whereas both advanced joint destruction and RA-ILD are related to JAK-STAT pathway (19, 20), these have not been reported as predictive factors for JAKis response. The pathogenic heterogeneity of RA in both genetic and epigenetic modifications is one of the main causes of the difference in the response to JAKis (21) and may explain the existence of a subpopulation with a combination of specific factors. In other words, JAK-STAT pathway dominant RA could be identified using a combination of seropositivity, advanced joint destruction and RA-ILD. The genetic variations of human leucocyte antigen (HLA) class II are associated with antigen presentation and autoantibody production (22). In particular, HLA DRB1 alleles that code "shared epitope" (SE) are associated with ACPA production, as well as advanced bone destruction (23, 24). JAKis has the ability to suppress osteoclastogenesis through downregulation for the receptor activator of nuclear factor-κB ligand (RANKL) expression by osteoblasts or reduction in RANKL production by T cells (20). SE would associate with JAK-STAT pathway dominant bone destruction. Conversely, SE would affect to lung involvement in RA, probably related with the low prevalence of RA-ILD (25). Lung tissues of RA-ILD rat model showed increased protein levels of JAK/STAT, suggesting that JAK/STAT signalling pathway is implicated in the RA-ILD as well as in arthritis (26). Other genetic and epigenetic modification may contribute to autoantibody production and RA-ILD development. In our study, the seronegative RA subpopulation (Group A) has more advanced joint destruction compared to the seropositive RA subpopulation (Group B and C). It was considered that patients in Group A had a tendency of longer disease duration than Group B and C, and joint destruction would be more progressive with longer disease affection (27).

The heterogeneity of RA-fibroblast-like synoviocytes (RA-FLS) might also be related to the response to JAKis. Different RA-FLS or fibroblast subsets have been identified by morphology, transcriptome and function analysis, leading to spatial heterogeneity with biological differences between various joints. Hammaker *et al*. (28) reported that knee FLS were less sensitive to tofacitinib than hip FLS in RA patients. In fact, RA hip and knee FLS have distinct transcriptomes, epigenetic marks, and STAT3 activation patterns in the IL-6 pathway. These joint-specificity of FLS may contribute to a differential clinical response to JAKis.

There are some major limitations to be considered in this study as follows: 1) This study comprised relatively low number of the patients and only Japanese population, leading to each cluster small sample size. We defined our study as a preliminary investigation and confirmatory studies in larger and global independent cohorts are required to validate this study. 2) Type of RA-ILD, such as UIP and NSIP, and joint specificity in bone destruction were not included in the analysis.

In conclusion, we identified a subpopulation of JAKis-IR using cluster analysis. Seronegative RA with destructive phenotypes but without lung disease may be less benefitted from JAKis treatment. Though it is still challenging to suggest the feasibility of personalised medicine for RA patients and previous clinical studies have failed to clarify the characteristics of patients with JAKis-IR, cluster analysis would be useful for identifying some subgroups of RA patients who share peculiar characteristics, including DMARD responsibility.

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