Circulating microRNAs associated with tumour necrosis factor or fibroblast-like synoviocytes predict cartilage damage in early rheumatoid arthritis treated with methotrexate plus adalimumab: a subanalysis of the MIRACLE study

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Abstract

Objective

This study aimed to explore the potential of plasma micro-ribonucleic acids (miRNAs) in predicting joint damage in patients with rheumatoid arthritis (RA).

Methods

This subanalysis of the MIRACLE study, a randomised, open-label, non-inferiority trial, explored and compared the efficacy and safety of treatment with adalimumab (ADA), an anti-tumour necrosis factor (TNF) α, plus a maximum tolerated dose of methotrexate (MTX) with a reduced dose of MTX in early RA. Plasma levels of miRNAs (miR-143-3p, miR-146a-5p, miR-155-5p, miR-182-5p, miR-21-5p, and miR-221-3p) and serum levels of inflammatory cytokines (interleukin-6 [IL-6], vascular endothelial growth factor [VEGF]) and matrix metalloprotease-3 (MMP-3) were measured at 24 weeks. Their association with joint destruction assessed by the modified total Sharp score [mTSS] over the 24-week period were analysed.

Results

A total of 134 patients who showed an inadequate response to MTX and started treatment with ADA were included in the analyses. Logistic regression analyses revealed that higher plasma levels of miR-143-3p, miR-146a-5p, miR-21-5p, and miR-221-3p were significantly associated with increases in mTSS >0.5 points during the observation period. In particular, positive correlation was derived from the progression of joint space narrowing. In contrast, MMP-3, VEGF, and IL-6 levels were not associated with joint destruction. Cartilage damage occurred mainly in patients treated with reduced dose of MTX.

Conclusion

Higher circulating miRNA levels predicted subsequent cartilage damage in early RA treated with a TNF inhibitor in addition to MTX. Thus, the MTX dose at ADA initiation should not be reduced in patients with high microRNA levels.

Key words

microRNAs, rheumatoid arthritis, biomarkers, cartilage

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Competing interests: see page 497.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterised by persistent synovitis, leading to joint destruction and physical dysfunction (1). The introduction of novel effective agents against RA and the application of treat-to-target (T2T) strategies in clinical practice have markedly improved the quality of life and prognosis of patients with RA. However, there remain intractable cases in which joint destruction progresses despite intensive therapies (2). In the PREMIER study, 1-year treatment with methotrexate (MTX) and adalimumab (ADA), a human anti-tumour necrosis factor α (TNF-α) monoclonal antibody, enabled the achievement of the American College of Rheumatology (ACR) 50 response in 62% of patients with early RA. However, 36% of the patients showed progressive joint destruction, with an increase in the total Sharp score (TSS) of more than 0.5 during the observation period (3). Accordingly, controlling disease activity in early RA is becoming a feasible objective in clinical practice with T2T strategies, though challenges remain in achieving structural remission due to the lack of established clinical indicators to guide treatment and strategies.

Micro-ribonucleic acids (miRNAs) are single-stranded, non-coding RNAs that regulate protein expression by inhibiting the translation of messenger RNAs. miRNA genes are estimated to comprise 1–2% of the total genome, and more than 2,000 miRNAs have been identified (4). Because alterations in miRNA expression regulate cell differentiation, proliferation, and apoptosis, miRNAs are deeply involved with the pathogenesis of various diseases. Furthermore, circulating miRNAs could be promising biomarkers in certain cases of cancer and cardiovascular diseases (5-6). Since the increased expression of miR-146a and miR-155 in inflammatory synovial tissues of patients with RA was first reported in 2008, several miRNA expressions in whole blood, plasma, peripheral blood mononuclear cells, synovial fluid, and synovial fibroblasts have been identified in RA (7). Previous studies revealed the contribution of miRNAs to the differentiation and proliferation of synoviocytes, osteoclasts, and chondrocytes, as well as the production of inflammatory cytokines (8-9). To date, several studies have investigated the potential of circulating miRNAs to predict disease activity outcomes in RA (10-18). However, no study has elucidated the association of miRNAs with joint destruction. Furthermore, joint space narrowing (JSN) appears to be more clearly associated with irreversible physical disability than bone erosion in RA, highlighting the importance of distinguishing between cartilage and bone damage in analysing joint destruction (19).

The objective of this study was to explore and compare the usefulness of plasma miRNAs for predicting joint destruction, cartilage damage, and bone erosion in patients with RA with other serum biomarkers including proinflammatory cytokines.

Materials and methods

Study design and patients

This was an exploratory subanalysis of the MIRACLE study, a multicentre, open-label, randomised, interventional study (ClinicalTrials.gov identifier NCT03505008) conducted in MTX-naive patients with RA in Japan, South Korea, and Taiwan (20-21). Details of the MIRACLE study design, participants, randomisation, masking, and procedures have been described in our previous report (20-21). Briefly, the enrolled patients were aged ≥18 years (≥20 years in Taiwan), and the diagnosis of RA was made according to the classification criteria proposed by the ACR revised in 1987 (22) or ACR-European Alliance of Associations for Rheumatology (EULAR) in 2010 (23). Patients were treated with a maximum tolerable dose of MTX, and those who did not achieve Simplified Disease Activity Index (SDAI) remission at week 24 despite MTX at a dose of more than 10 mg per week were randomised to ARM-2 (continued maximum tolerable MTX dose) or ARM-3 (reduced MTX dose) and started on ADA. This study was approved by the Ethics Committee of each participating institution and was performed in accordance with the tenets of the Declaration of Helsinki. Written

informed consent was obtained from each participant before participation in the trial.

This study included patients who were allocated to the ARM-2 or ARM-3 in the MIRACLE study and planned to have their plasma miRNA levels measured at week 24, which was considered as the study baseline, to investigate the differences in joint damage between two treatment types. Serum or plasma levels of cytokines (interleukin-6 [IL-6]: serum, vascular endothelial growth factor [VEGF]), and matrix metalloproteinase-3 (MMP-3) levels were also measured at weeks 0, 24, and 48.

Data collection and definition

We collected the data, including patient characteristics, disease activity, and modified TSS (mTSS) composed of bone erosion (Erosion) or JSN scores (24). The x-ray images of the hands and feet were independently reviewed by the X-ray Imaging Review Committee to evaluate bone erosions and joint space narrowing in terms of the mTSS, and the results were sent to the data centre. Structural remission and clinically relevant radiographic progression were defined as a change in the mTSS (ΔmTSS) ≤0.5 and >3, respectively.

Sample measurement

Six candidates of miRNAs, miR-143-3p (17), miR-146a-5p (25), miR-155-5p (26), miR-182-5p (27), miR-21-5p (28), and miR-221-3p (29), were selected based on previous reports, and plasma levels of these miRNAs were measured at week 24 in this study. For the measurement of plasma miRNAs, 3 mL of blood samples were centrifuged at room temperature at 16,000×g for 10 minutes, and plasma was stored at -80°C. NucleoSpin® miRNA Plasma Kit (Macherey-Nagel, Düren, Germany) was used to extract total RNA, which includes miRNA from 200 µL plasma samples. When the volume of plasma was insufficient, RNase-free H2O was applied to 200 µL. A volume of 50 µL RNase-free rDNase was added to the column into which the RNA was eluted. Total RNA samples were kept at -80°C until use. cDNA was synthesised using the miRCURY LNA® RT Kit (Qiagen, Hilden, Germany). Each reverse transcription reaction volume was 10 µl using 2.0 µl 5×miRCURY RT Reaction Buffer, 5.0 µl Nuclease-free water, 1.0 µl 10×miRCURY RT Enzyme mix, and 2.0 µl total RNA sample. cDNA synthesis was performed using the Ge-neAmp® polymerase chain reaction (PCR) System 9700 (Life Technologies, Foster City, CA, USA) (42°C, 60 min; 95°C, 5 min; hold at 4°C). Real-time PCR was performed using the miRCURY LNA SYBR® Green PCR kit (Qiagen, Hilden, Germany) and GeneAmp® PCR System 9700. The following six miRNAs were measured using PCR: hsa-miR-143-3p with the sequence 5'UGAGAUGAAGCACUGUAGCUC, hsa-miR-146a-5p with the sequence 5' UGAGAACUGAAU-UCCAUGGGUU ', hsa-miR-155-5p with the sequence 5' UUAAUGCUAAUCGUGAUAGGGGU, hsa-miR-182-5p with the sequence 5'UUUGGCAAUGGUAGAACUCACACU, hsa-miR-21-5p with the sequence 5' UAGCUUAUCAGACUGAUGUUGA, and hsa-miR-221-3p with the sequence 5'AGCUACAUUGUCUGCUGGGUUUC. Each SYBR Green master mix reaction was 10 µl consisting of 5.0 µl of 2×miRCURY SYBR Green PCR Master Mix, 1.0 µl of Nuclease free water, 1.0 µl of LNA PCR Primer mix, and 3.0 µl of each sample and standard. The PCR cycling conditions were as follows: initial denaturation at 95°C for 2 min, followed by 45 cycles of denaturation at 95°C for 10 s, and annealing at 56°C for 1 min.

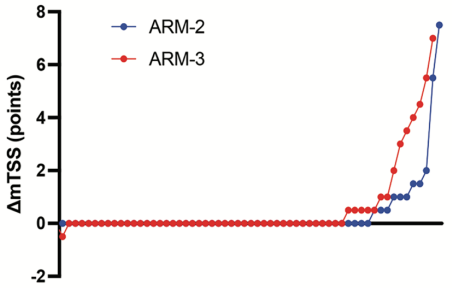
IL-6 and VEGF levels were measured from EDTA plasma using a chemiluminescent enzyme immunoassay with Quanti Glo™ ELISA Human IL-6 Immunoassay Kit (R&D Systems, Minneapolis, MN, USA) and an enzyme immunoassay with Quantikine® Human VEGF Immunoassay Kit (R&D Systems, Minneapolis, MN, USA), respectively. The MMP-3 test, which is covered by health insurance in Japan, was performed on serum using a latex agglutination turbidimetric immunoassay.

Statistical analysis

For the statistical analysis, we employed an available case analysis approach, where only complete cases without missing values were included. Continuous variables are presented as mean ± standard deviation (SD) for normally distributed data or median and interquartile range (IQR) for non-normally distributed data. Categorical variables are expressed as counts and proportions.

Comparisons of continuous variables between the two groups were performed using the Mann-Whitney U-test for non-normally distributed data, while the Kruskal-Wallis test was applied for comparisons across more than two groups. Categorical variables were analysed using Fisher's exact test or Pearson's Chi-square test. Logistic regression models were applied to assess the association between baseline char-

A Cumulative probability plot of change in mTSS



B

ΔmTSS (24 – 48w)	ARM-2 (N=59)	ARM-3 (N=58)	p
mean	0.38	0.58	0.58
≤ 0, N (%)	48 (81.4)	44 (75.9)	0.47
> 0.5, N (%)	8 (13.6)	9 (15.5)	0.76
> 3, N (%)	2 (3.4)	5 (8.6)	0.23

Fig. 1. Radiographic progression of joint damage over the period from week 24 to 48.
A: Cumulative probability plots of change in mTSS from week 24 to 48.
B: The numbers (%) of patients who achieved no radiographic progression, radiographic remission, and clinically relevant radiographic progression in ARM-2 and ARM-3.
mTSS: modified total Sharp score.

acteristics and the likelihood of structural non-remission (ΔmTSS >0.5). In the multivariate analysis, adjusted odds ratios (ORs) with 95% confidence intervals (CI) were estimated to account for potential confounders, including treatment arms (ARM-2 or ARM-3), baseline positivity for rheumatoid factor (RF) and anti-citrullinated cyclic peptide (anti-CCP) antibodies, and SDAI at week 24.

The correlation between plasma miRNA levels and mTSS scores was evaluated using Spearman's rank correlation coefficient (ϱ). Diagnostic performance of miRNAs in predicting joint destruction was further evaluated by constructing receiver operating characteristic (ROC) curves, with cut-off values determined using the Youden index to maximise sensitivity and specificity. All the statistical tests were two-sided, and a *p*-value of less than 0.05 was considered statistically significant. Analyses were performed using JMP Pro 16 (SAS Institute Inc., Cary, NC, USA) for regression models and descriptive statistics, and GraphPad Prism 10.0.2 (GraphPad Software, La Jolla, CA, USA) for ROC curve analysis.

Results

Characteristics of enrolled patients at baseline and at week 24

A total of 134 patients who did not achieve SDAI remission at week 24 in the MIRACLE trial were included in the current analyses, consisting of 68 patients treated with ADA in combination with the maximum tolerated dose of MTX (the ARM-2 group) and 66 patients treated with ADA in combination with a reduced dose of MTX (the ARM-3 group). Among them, 9 patients (13%) in the ARM-2 group (3 due to consent withdrawal, 3 due to arthritis activity, 2 due to adverse events, and 1 due to methotrexate reduction) and 8 (12%) in the ARM-3 group (3 due to consent withdrawal, 2 due to adverse events, 2 lost to follow-up, and 1 due to arthritis activity) discontinued the study before the end of the trial (week 48). The levels of RF and anti-CCP antibodies were higher in the ARM-3 group than in the ARM-2. However, other clinical variables at baseline were not significantly different between the two ARMs (Supplementary Table S1). SDAI, MMP-3, IL-6, and VEGF at baseline and week 24 as well as miRNA levels at week 24 were comparable between the two ARMs (Suppl. Table S2).

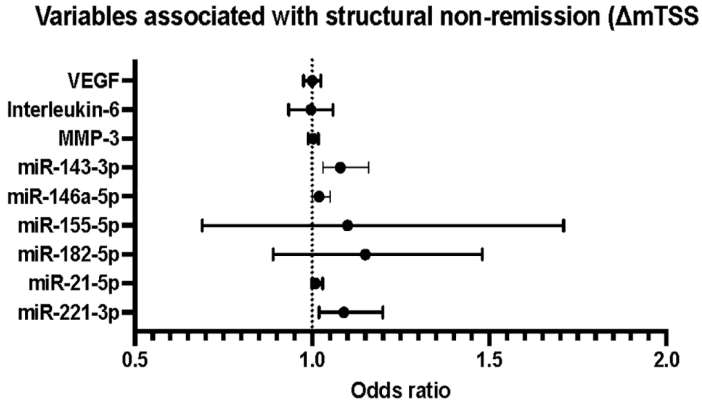


Fig. 2. Comparison of predictive potential of joint damage by miRNAs and other markers.
Association of miRNAs and other markers at week 24 with structural damage progression during the 24-week period from week 24 to 48 evaluated using changes in mTSS adjusted by ARM, RF positivity (week 0), Anti-CCP antibody positivity (week 0), and SDAI (week 24).
miRNA: micro ribonucleic acid; mTSS: modified total Sharp score; RF: rheumatoid factor; CCP: cyclic citrullinated peptide.

Table I. Single regression analyses between miRNAs and structural changes of joints after 24 weeks of treatment.

	ARM-2 (n=59)		ARM-3 (n=58)	
modified total Sharp score				
	rs (ϱ)	*p*-value	rs (ϱ)	*p*-value
miR-143-3p	0.02	0.87	0.28	**0.03**
miR-146a-5p	-0.07	0.60	0.23	0.09
miR-155-5p	-0.10	0.46	-0.01	0.94
miR-182-5p	-0.04	0.75	-0.03	0.85
miR-21-5p	0.03	0.85	0.30	**0.02**
miR-221-3p	0.02	0.88	0.17	0.20
Erosion score of modified total Sharp score				
	rs (ϱ)	*p*-value	rs (ϱ)	*p*-value
miR-143-3p	-0.11	0.39	0.07	0.62
miR-146a-5p	-0.12	0.35	0.12	0.34
miR-155-5p	-0.03	0.83	0.10	0.44
miR-182-5p	-0.08	0.53	0.14	0.29
miR-21-5p	-0.05	0.72	0.17	0.20
miR-221-3p	0.002	0.99	0.10	0.44
Joint space narrowing score of modified total Sharp score				
	rs (ϱ)	*p*-value	rs (ϱ)	*p*-value
miR-143-3p	0.27	**0.04**	0.37	**0.01**
miR-146a-5p	0.07	0.59	0.30	**0.02**
miR-155-5p	-0.03	0.81	-0.03	0.83
miR-182-5p	0.07	0.58	-0.05	0.72
miR-21-5p	0.20	0.13	0.32	**0.01**
miR-221-3p	0.09	0.51	0.27	**0.04**

miRNAs are measured at week 24. RNA: ribonucleic acid; miR: micro RNA.

Association between plasma miRNA levels and joint destruction

Fig. 1A and B show the cumulative probability plots of change in mTSS and proportion of patients achieving structural remission or radiographic

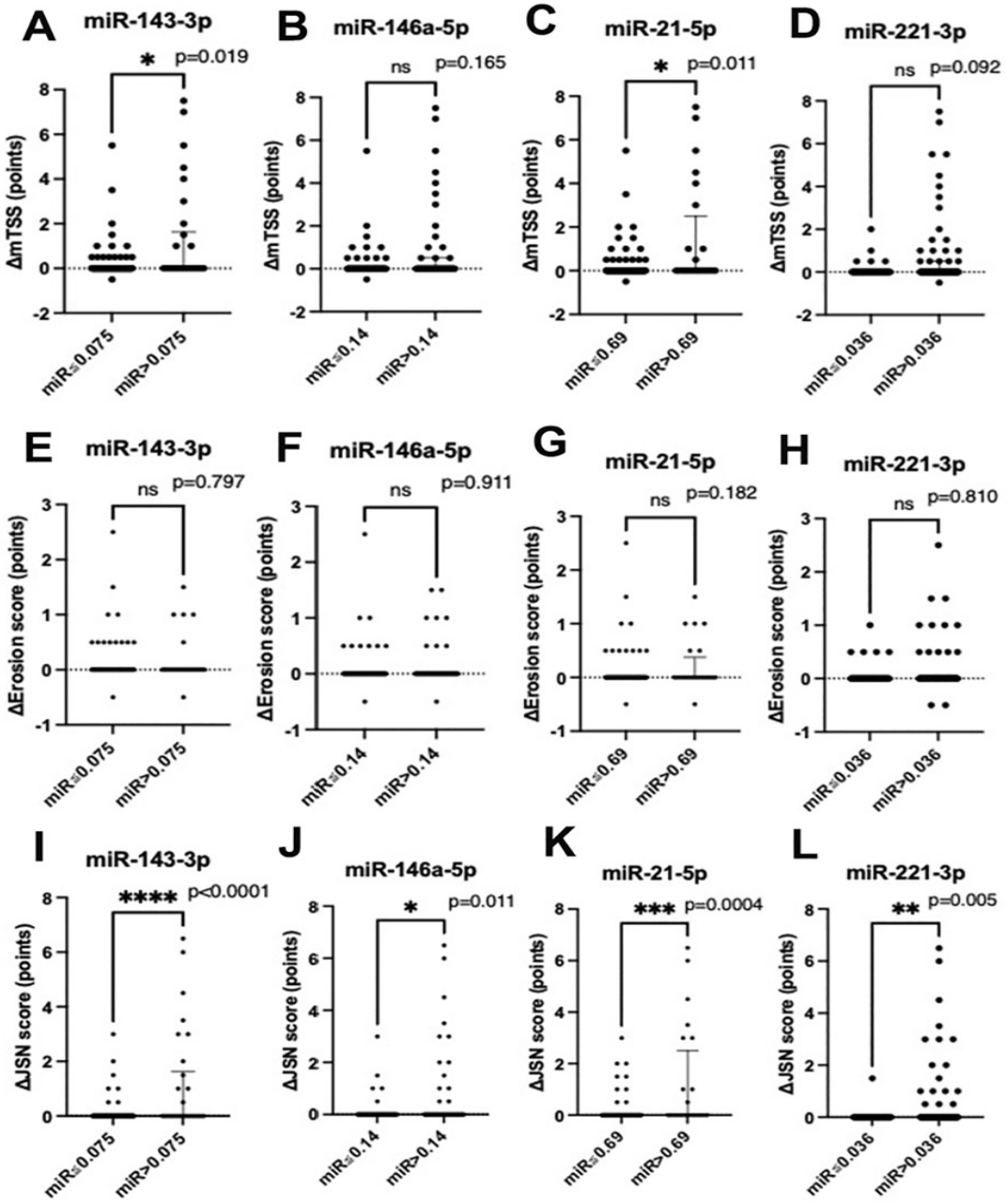


Fig. 3. Differences in the progression of joint damages stratified by a cut-off level of each miRNA.
Differences in the progression of joint damages divided by a cut-off level of each miRNA at week 24. Progression of joint damage evaluated by **A-D**: mTSS, **E-H**: erosion score, and **I-L**: JSN score.
miRNA: micro ribonucleic acid; mTSS; modified total Sharp score; JSN: joint space narrowing.

progression. These proportions were similar between the two ARMs despite different MTX dosages.

In the univariate logistic regression analysis, anti-CCP antibody positivity at baseline, and SDAI, MMP-3 levels, miR-143-3p, miR-146a-5p, miR-21-5p, and miR-221-3p levels at week 24 were positively associated with structural non-remission (ΔmTSS >0.5) (Suppl. Table S3). In the multivariable analysis, higher plasma miR-143-3p, miR-146a-5p, miR-21-5p, and miR-221-3p levels were positively associated with structural non-remission. In contrast, MMP-3, VEGF, and IL-6 levels at week 24 were not associated with joint destruction. Notably, plasma miR-143-3p and miR-221-3p levels were predictive of structural non-remission with adjusted odds ratios of 1.08 (95% CI: 1.03, 1.16) and 1.09 (95% CI: 1.02, 1.20), respectively (Suppl. Table S3, Fig. 2).

Interestingly, significant correlations between these microRNAs and joint destruction were observed particularly in the ARM-3 and derived from the JSN scores, but not in the erosion scores (Table I).

Prediction of joint destruction with each miRNA and the combination of miRNAs

ROC curves revealed that plasma miR-143-3p, miR-146a-5p, miR-21-5p, and

miR-221-3p levels were predictive of structural non-remission, with cut-off values of 0.075, 0.14, 0.69, and 0.036, respectively. Supplementary Table S4 summarises the sensitivity and specificity of each microRNA; high sensitivity for miR-143-3p (80.0%) and miR-21-5p (84.0%), and high specificity for miR-221-3p (88.2%). When patients were stratified by these cut-off values, those in the higher levels of miR-143-3p, miR-146a-5p, miR-21-5p, or miR-221-3p groups showed significantly higher changes in the JSN scores, but not in the erosion scores (Fig. 3). Furthermore, we investigated the combined predictive power of miRNAs levels. The combination of miR-143-3p >0.075 and miR-221-3p >0.036, as well as the combination of miR-21-5p >0.69 and miR-221-3p >0.036, strongly predicted future progression of joint space narrowing (Fig. 4).

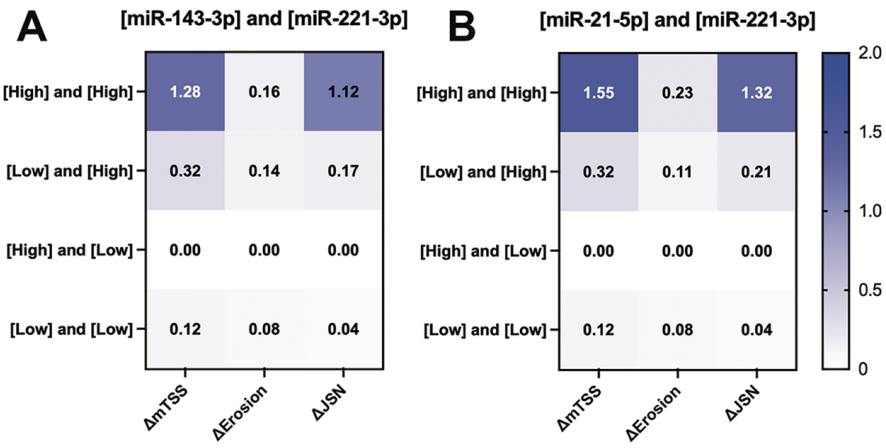


Fig. 4. Differences in joint damage progression stratified by the combination of two miRNA levels. Differences in joint damage progression based on two miRNA cut-off levels at week 24. ΔmTSS, ΔErosion, and ΔJSN indicate mean changes in progression of structural damage during the 24-week period from week 24 to 48.
A: The combination of miR-143-3p and miR-221-3p with cut-off levels of 0.075 and 0.036.
B: The combination of miR-21-5p and miR-221-3p with cut-off levels of 0.69 and 0.036.
miRNA: microribonucleic acid; mTSS; modified total Sharp score; JSN: joint space narrowing.

Discussion

This study revealed that higher plasma miR-143-3p, miR-146a-5p, miR-21-5p, and miR-221-3p levels were predictive of the subsequent joint destruction, especially cartilage damage, even with additional treatment with an anti-TNFα in early RA who had been inadequately responsive to MTX. Importantly, these miRNA levels were significantly correlated with cartilage damage in patients who were treated with a reduced dose of MTX.

There have been few reports on the association between microRNAs and radiographic joint damage in RA. Cuppen *et al*. found that higher levels of miR-143 at baseline were observed in non-responders among patients with RA starting ADA in an observational cohort (17). The upregulated miR-143 can downregulate insulin-like growth factor binding protein 5 and render fibroblast-like synoviocytes (FLSs) susceptible to TNF-α in RA (30). This might contribute to the inadequate response to TNF inhibitors, leading to a long-standing inflammation with cartilage damage in the affected joints. Pauley *et al*. demonstrated that high levels of miR-146a expression in peripheral blood mononuclear cells from RA patients correlated with disease activity of RA (25). Although TNF receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRAK-1) are established targets of miR-146a, the expression levels of TRAF6/IRAK-1 were similar between patients with RA and healthy individuals. Jin *et al*. reported that miR-21 upregulated, by Maresin 1 reduced joint inflammation and improved the imbalanced Treg/Th17 ratio, ameliorating the RA progression (28). These facts indicate that miR-146a or miR-21 expression is increased in RA but unable to properly regulate TRAF6/IRAK-1 or Treg/Th17 balance, leading to prolonged inflammation in RA. On the other hand, the overexpression of miR-221 in RA with high disease activity was reported by Abo *et al*. (29). The miR-221 suppressed osteoblast differentiation in the FLS of C57BL/6 mice transferred with serum from RA model mice (K/BxN) (31). This study suggests that miR-221 secretion is upregulated in TNF-treated FLS, inhibiting the Wnt signalling pathway and contributing to the suppression of osteoblast maturation and bone formation in erosions. In addition, miR-221 has been implied to suppress p27 and p57 expression in various cancer cell lines, which are known to suppress the proliferation of osteoblasts and chondrocytes (32-33). These mechanisms could support our finding of high specificity for miR-221 in predicting cartilage damage in RA.

Interestingly, the correlation between miRNAs and the JSN score was stronger in patients assigned to the reduced dose MTX group than in those assigned to the maximum tolerated MTX dose group. Although the suppression of disease activity and joint damage was comparable between the maximum tolerated dose and reduced dose MTX groups in the whole population in the MIRACLE trial, our current data imply that the dose of MTX should not be reduced at ADA initiation in patients who have higher levels of the above-mentioned miRNAs to minimise the risk of cartilage damage. In the future, functional analysis of the candidate miRNAs would be valuable for determining whether inhibition of these miRNAs leads to suppression of joint damage.

Our study had certain limitations. First, measurements of miRNA were conducted only once at week 24 after initiating MTX per protocol. Therefore, we were unable to assess changes in plasma miRNA levels over time. However, all patients included in this study had their miRNA levels evaluated at the same point of starting ADA to MTX, which enabled the assessment of the association of microRNAs and sequential therapeutic response. Second, the study population was limited to Asians, which may limit the generalisability of our findings. Third, miRNA measurements in this study were performed

using plasma samples, but it is unclear whether the values obtained reflect local inflammation in the joints. However, several studies have found the association of plasma miRNA with clinical manifestations or responsiveness to RA treatment to date (34-36).

In conclusion, our study suggests that plasma miRNAs can predict cartilage damage in patients with early RA and provides insights to understand the pathogenesis of osteocartilage degradation in future.

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Competing interests

Y. Hosokawa received honorarium from Eisai. Y. Yoshida received honoraria from Eisai, Asahi Kasei, Daiichi-Sankyo and Astellas. H. Tamai received honoraria from AbbVie, Asahi Kasei, Eisai and Novartis; support for attending meetings from PhRMA. S. Hirata received grants from AbbVie, Asahi-Kasei, Chugai, Eisai, Eli Lilly, Otsuka, Taisho and UCB; honoraria from AbbVie, Asahi Kasei, Astellas, AstraZeneca, Ayumi, Bristol Myers Squibb, Boehringer Ingelheim, Chugai, Daiichi-Sankyo, Eisai, Eli Lilly, Gilead Sciences, Glaxo Smithkline, Janssen, Nippon Shinyaku, Novartis, Otsuka, Pfizer, Taisho, Mitsubishi-Tanabe and UCB; participated on advisory boards of AbbVie. K. Ikeda received grants from Mitsubishi-Tanabe; honoraria from AbbVie, Eisai, Pfizer, Mitsubishi-Tanabe, Janssen, Novartis, UCB, Daiichi-Sankyo, Eli Lilly, Gilead Sciences, AstraZeneca, Asahi Kasei, Chugai, Bristol Myers Squibb. T. Miyamoto received consulting fees from Eisai, Gilead Sciences; honoraria from Eisai, AbbVie, Eli Lilly, Gilead Science, Asahi Kasei. K. Shin received grants/research support from Novartis, Astellas, AbbVie, Bristol Myers Squibb, Yuhan; consulting fees from KD Bio. K. Izumi received grants from AbbVie; honoraria from Eisai. Y. Kondo received speaking fees from AbbVie, Ayumi Pharmaceutical Corporation, Boehringer Ingelheim, Bristol-Myers Squibb, Chugai, Eisai, Eli Lilly, Glaxo Smith Kline, Gilead Sciences, Pfizer and Taisho. H. Yasuoka received grants from AbbVie, Boehringer Ingelheim, Gilead Sciences, Nippon Shinyaku, Asahi Kasei, Chugai, Eisai, Mitsubishi-Tanabe, Taisho; honoraria from AbbVie, Asahi Kasei, Astellas, Daiichi Sankyo, Eisai, Eli Lilly, Pfizer, Janssen, Sanofi, Teijin, Boehringer Ingelheim, Bayer, Viatris, Kissei, Takeda, Mitsubishi-Tanabe, Chugai, Novartis. MK received grants and royalties from MBL; consulting fees from Boehringer Ingelheim, Mochida, Kissei, GSK, AstraZeneca, Mitsubishi-Tanabe, Janssen, Biogen; received honoraria from Boehringer Ingelheim, Chugai, Asahi Kasei; participated on advisory board of Argenx. T. Ishii received honoraria from Asahi Kasei, GSK, Chugai, Pfizer, Eli Lilly, Boehringer-Ingelheim, AstraZeneca. H. Kameda received a grant from Pfizer, AbbVie, Asahi Kasei, Boehringer Ingelheim, Chugai, Eisai, Mitsubishi-Tanabe, Taisho; consulting fees from Sanofi; honoraria from AbbVie, Asahi Kasei, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Chugai, Eisai, Eli Lilly, Janssen, Mitsubishi-Tanabe, Novartis, Pfizer. T. Kojima received grant/research support from AbbVie, Astellas, Chugai; honoraria from AbbVie, Astellas, Bristol-Myers Squibb, Chugai, Daiichi-Sankyo, Eli Lilly, Pfizer, Eisai, Taisho, UCB; participated on board of JCR subcommittee on Guidelines for RA Therapeutics. M. Mori is a shareholder and employee of Eisai. Y. Sato received honoraria from Mochida, AstraZeneca K.K., SBI Pharmaceuticals Co., Ltd., Janssen Pharmaceutical K.K. W. Tsai received consulting fees and honoraria from Eli Lilly and Eisai; honorarium from Pfizer. T. Takeuchi received consulting fees from AbbVie, Eli Lilly, Gilead Sciences, Mitsubishi-Tanabe, Taisho; honoraria from AbbVie, Astellas, AstraZeneca, Chugai, Eisai, Eli Lilly, Gilead Sciences, Janssen, Mitsubishi-Tanabe, Pfizer, Taisho. Y. Kaneko received grants from AbbVie, Asahi-Kasei, Ayumi, Boehringer Ingelheim, Chugai, Eisai, Gilead Sciences, Mitsubishi-Tanabe, Taisho, UCB; consulting fees from AbbVie, Asahi Kasei, Bristol Myers Squibb, Eli Lilly, Gilead Sciences, Pfizer, Taisho, UCB; hono-

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