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RESEARCH ARTICLE

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The potential of circulating microRNA-125a and microRNA-125b as markers for inflammation and clinical response to infliximab in rheumatoid arthritis patients

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Abstract

Objective: This study was to investigate the changes in circulating microRNA (miR)-125a and miR-125b during infliximab (IFX) treatment, and their value in predicting clinical response to IFX in rheumatoid arthritis (RA) patients.

Methods: The plasma samples were obtained from 96 active RA patients who underwent 24-week IFX treatment and from 96 healthy controls to detect miR-125a and miR-125b expressions by RT-qPCR. Clinical response was assessed according to EULAR criteria based on disease activity alleviation at week 4, week 12, and week 24.

Results: MiR-125a and miR-125b expressions were both elevated in RA patients compared with healthy controls, and they could differentiate RA patients from healthy controls by receiver operating characteristic curve analysis. Baseline miR-125a positively correlated with C-reactive protein (CRP) level; meanwhile, baseline miR-125b positively correlated with tender joint count (TJC), swollen joint count (SJC), erythrocyte sedimentation rate (ESR), CRP, and DAS28-ESR score in RA patients. With the 24-week IFX treatment, clinical response rate was gradually increased, while miR-125a and miR-125b expressions were gradually decreased in RA patients. At week 24, 69 (71.9%) patients responded to IFX treatment, while 27 (28.1%) patients did not respond to IFX treatment. Importantly, baseline miR-125a and miR-125b expressions were higher in responders than that in non-responders, further multivariate logistic regression analysis disclosed that miR-125b but not miR-125a could independently predict better clinical response to IFX in RA patients.

Conclusion: Circulating miR-125a and miR-125b displays the potency for guiding personalized treatment strategy and improving clinical outcomes in RA patients.

KEYWORDS

clinical response, infliximab, microRNA-125a, microRNA-125b, rheumatoid arthritis

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease, which is marked by progressive joint destruction and associated systemic manifestations in vascular, metabolic, bone, and psychological domains.[1] In recent years, the expanded therapeutic options of conventional disease-modifying antirheumatic drugs, tumor necrosis factor (TNF) inhibitor, and other biological agents have improved both the management and long-term prognosis of RA.[2] Infliximab (IFX), a TNF inhibitor, is a genetically constructed immunoglobulin G1 murine-human chimeric monoclonal antibody, which has been proven to alleviate disease activity, improve physical function, and slow radiographic damage in RA patients.[3] Unfortunately, approximately 20% to 40% of patients discontinue IFX treatment due to inefficacy, adverse events, or high cost in a real-life setting.[4,5] Thereby, the exploration of biomarker for the prediction of clinical response to IFX is paramountly important to optimize treatment strategy and improve clinical outcomes in RA patients.

MicroRNA (MiR)-125a and miR-125b have been proposed as important regulators for innate immune and inflammatory responses in various inflammatory diseases including RA.[6-9] In RA, plasma level of miR-125a is elevated in RA patients compared with control subjects, and it exhibits a good value in assessing RA risk.[7] As for miR-125b, it facilitates inflammation through activating nuclear factor kappa B (NF-κB) in RA.[9] Clinically, serum level of miR-125b is increased in RA patients compared with osteoarthritic and healthy donors, and it predicts good clinical response to rituximab treatment in RA patients.[8] On the basis of the above data, we hypothesized that miR-125a and miR-125b display the potential as biomarkers for assessing disease activity and clinical response to IFX in RA patients. But to date, no related study has been explored yet. Therefore, the present study was to investigate the changes in circulating miR-125a and miR-125b during IFX treatment, and the value of circulating miR-125a and miR-125b in predicting clinical response to IFX in RA patients, aiming to facilitate the treatment decision-making and disease management of RA in clinical setting.

2 | MATERIALS AND METHODS

2.1 | Patients

This prospective study consecutively enrolled 96 active RA patients about to receive IFX treatment in the Central Hospital of Wuhan. The recruiting period was ranging from January 2016 to December 2018. The inclusion criteria were as follows: (a) diagnosed as RA according to 2010 Rheumatoid arthritis classification criteria [10]; (b) age between 18 and 80 years old; (c) disease activity score in 28-joint count-erythrocyte sedimentation rate (DAS28-ESR score) ≥3.2 (active disease status); (d) planed and willing to receive IFX combined with single conventional disease-modifying antirheumatic drug (cDMARD) treatment at least for 6 months; (e) history of at least single/multiple cDMARDs treatment. The exclusion criteria were as follows: (a) underwent glucocorticoid therapy within one month; (b) severe deformation of joint; (c) known poor clinical response to IFX or intolerance to IFX; (d) history of hematological malignancies, solid tumors, severe infections or other immune rheumatism diseases; (e) unable to be followed up regularly, which was assessed by their attending physicians. In addition, 96 age- and gender-matched healthy subjects (age was limited within 45-75 years old, and gender was matched in 1:3 (male: female) on enrollment) were recruited as healthy controls at the same period. The Institutional Review Board of the Central Hospital of Wuhan approved this study. All patients signed the informed consents before enrollment.

2.2 | Baseline data collection

The demographics (such as age, gender, body mass index (BMI)), medical history (such as disease duration, history of biologics, history of cDMARDs), serum markers (eg, rheumatoid factor (RF) status or anti-citrullinated protein antibodies (ACPA) status), disease activity indexes (such as tender joint count (TJC), swollen joint count (SJC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, health assessment questionnaire disability index (HAQ-DI) score), and combined medications (eg, methotrexate (MTX) or leflunomide (LEF)) of patients were collected after enrollment. And DAS28-ESR score was calculated according to TJC, SJC, and ESR level, and the formula was as follows: $\mathrm{DAS28-ESR} = [0.56\sqrt{\mathrm{TJC}} + 0.28\sqrt{\mathrm{SJC}} + 0.70 * \ln(\mathrm{ESR})] * 1.08 + 0.16.$

2.3 | 1.1. Sample collection

After enrollment, peripheral blood (PB) samples were collected at baseline (W0), week 4 (W4), week 12 (W12), and week 24 (W24). Then, the PB samples were centrifugalized at 3000 *g* for 20 minutes under 4°C. Subsequently, the plasma was separated and stored at −80°C for further detection.

2.4 | Treatment and assessment

All patients received IFX treatment as follows: intravenous injection of 3 mg/kg IFX at W0, week 2 (W2), and week 6 (W6), followed by the same dosage every 8 weeks. And the patients received IFX treatment at least for 24 weeks. In addition, 50 patients combined with MTX treatment and 46 patients combined with LEF treatment as follows: 10-20 mg MTX orally once a week or 10 mg LEF orally per day. Besides, DAS28-ESR score was calculated at W0, W4, W12, and W24 for assessment of clinical response. According to the European League Against Rheumatism (EULAR) response criteria, clinical response was defined as a change of 1.2 points in DAS28-ESR score from W0.[11] And all patients were classified as responder and non-responder based on clinical response at W24. Of note, the time

points for the administration of each dose of IFX were set according to clinical needs and medicine instruction, while clinical response was generally assessed every 3 months, thus, the time points for the administration of each dose of IFX were different from the time points for clinical assessment, although it decreased the execution efficiency.

2.5 | MiR-125a and miR-125b

The expressions of miR-125a and miR-125b in plasma samples at W0, W4, W12, and W24 were detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Initially, total RNA was extracted from plasma samples using QIAamp RNA Blood Mini Kit (Qiagen, Duesseldorf, Nordrhein-Westfalen, German), and the extracted total RNA was used for complementary DNA (cDNA) synthesis by ReverTra Ace® qPCR RT Kit (Toyobo). Then, RT-qPCR was performed using THUNDERBIRD® SYBR® qPCR Mix (Toyobo). The relative expressions of miR-125a and miR-125b were computed by $2^{-\triangle\triangle Ct}$ method with U6 as internal reference. The primers applied in the present study were shown as below: miR-125a, forward: 5′-ACACTCCAGCTGGGTCCCTGAGACCCTTTAAC-3′, reverse: 5′-TGTCGTGGAGTCGGCAATTC-3′;miR-125b,forward:5′-ACACTCCAGCTGGGTCCCTGAGACCCTAACTT-3′,reverse:5′-TGTCGTGGAGTCGGCAATTC-3′; U6,forward: 5′-CTCGCTTCGGCAGCACATATACTA-3′, reverse: 5′-ACGAATTTGCGTGTCATCCTTGC-3′.

2.6 | Statistical analysis

Based on the intention-to-treat (ITT) principles, the patients who early dropped out from this study (due to early losing follow-up, changing treatment regimen, poor efficacy or adverse events) were analyzed using the last observation carried forward (LOCF) method. Statistical analyses were performed with the use of SPSS 24.0 (IBM), and figures were plotted using GraphPad Prism 7.00 (GraphPad Software). Continuous variables were presented as mean ± standard deviation (SD) and interquartile range (IQR). Categorical variables were displayed as count (percentage). Comparison of miR-125a/b between two groups was determined by Wilcoxon rank-sum test. Comparisons of miR-125a/b between W0 and W4/W12/W24 were determined by Wilcoxon signed-rank test. Correlation of miR-125a/b with clinical characteristics was determined by Spearman's rank correlation test or Wilcoxon rank-sum test. Factors predicting clinical response at W24 were analyzed by univariate logistic regression model, and the factors with *P* value <.05 in univariate logistic regression were further analyzed in forward stepwise multivariate logistic regression for screening independent predictors. The screened independent predictors were used to construct the predictive model for clinical response (W24), and the formula was as follows: *P* = e^ (0.147 + 0.617 miR-125b-0.277 disease duration-1.447 history of biologics + 0.033

TABLE 1 Baseline characteristics of RA patients

Items	RA patients (N = 96)
Demographics	
Age (y)	
Mean ± SD	58.6 ± 10.0
Median (IQR)	57.5 (51.0-67.0)
Gender, No. (%)	
Male	19 (19.8)
Female	77 (80.2)
BMI (kg/m^2)	
Mean ± SD	22.5 ± 3.0
Median (IQR)	22.2 (20.4-24.9)
Medical history	
Disease duration (years)	
Mean ± SD	4.7 ± 3.5
Median (IQR)	3.5 (1.8-7.1)
History of biologics, No. (%)	18 (18.8)
History of cDMARDs, No. (%)	96 (100.0)
Serum markers	
RF status, No. (%)	
Negative	20 (20.8)
Positive	69 (71.9)
Unknown	7 (7.3)
ACPA status, No. (%)	
Negative	20 (20.8)
Positive	60 (62.5)
Unknown	16 (16.7)
Disease activity indexes	
TJC	
Mean ± SD	8.2 ± 3.2
Median (IQR)	7.0 (6.0-10.0)
SJC	
Mean ± SD	7.1 ± 3.6
Median (IQR)	6.0 (5.0-9.0)
ESR (mm/h)	
Mean ± SD	45.1 ± 24.7
Median (IQR)	41.7 (24.5-65.4)
CRP (mg/L)	
Mean ± SD	40.6 ± 32.4
Median (IQR)	28.5 (14.9-61.1)
DAS28-ESR score	
Mean ± SD	5.4 ± 0.7
Median (IQR)	5.5 (4.9-5.8)
HAQ-DI score	
Mean ± SD	1.7 ± 0.3
Median (IQR)	1.7 (1.6-1.9)

(Continues)

TABLE 1 (Continued)

Items	RA patients (N = 96)
Combined medications, No. (%)	
MTX	50 (52.1)
LEF	46 (47.9)

Abbreviations: ACPA, anti-citrullinated protein antibodies; BMI, body mass index; cDMARDs, conventional disease-modifying antirheumatic drugs; CRP, C-reactive protein; DAS28-ESR score, disease activity score in 28-joint count-erythrocyte sedimentation rate; ESR, erythrocyte sedimentation rate; HAQ-DI, health assessment questionnaire disability index; IQR, interquartile range; LEF, leflunomide; MTX, methotrexate; RA, rheumatoid arthritis; RF, rheumatoid factor; SD, standard deviation; SJC, swollen joint count; TJC, tender joint count.

CRP) / (1 + e^ (0.147 + 0.617 miR-125b-0.277 disease duration-1.447 history of biologics + 0.033 CRP)), −2Ln(L) = 101.500. And predicting performances of the predictive model and the independent predictors were further validated by plotting receiver operating characteristic (ROC) curve and area under the curve (AUC) with 95% confidence interval (CI). *P* value <.05 was considered as significant.

3 | RESULTS

3.1 | RA patients' characteristics

The mean age of RA patients was 58.6 ± 10.0 years (Table 1). There were 19 (19.8%) males and 77 (80.2%) females. The mean BMI of RA patients was 22.5 ± 3.0 kg/m^2. Regarding medical history, the mean disease duration was 4.7 ± 3.5 years; history of biologics and history of cDMARDs were found in 18 (18.8%) and 96 (100.0%) patients, respectively. As for disease activity indexes, the mean TJC, mean SJC, mean ESR, mean CRP, mean DAS28-ESR score, and mean HAQ-DI score were 8.2 ± 3.2, 7.1 ± 3.6, 45.1 ± 24.7 mm/h, 40.6 ± 32.4 mg/L, 5.4 ± 0.7, and 1.7 ± 0.3, respectively. Other detailed characteristics were exhibited in Table 1.

3.2 | The value of miR-125a and miR-125b in discriminating RA patients from healthy controls

MiR-125a (*P* < .001) (Figure 1A) and miR-125b (*P* < .001) (Figure 1B) relative expressions were elevated in RA patients compared with healthy controls. Subsequent ROC curve analyses reported that miR-125a (AUC: 0.832, 95% CI: 0.776-0.888) and miR-125b (AUC: 0.872, 95% CI: 0.864-0.921) were with good values in differentiating RA patients from healthy controls (Figure 1C). Besides, miR-125a positively correlated with miR-125b in both healthy controls (*P* = .001, r = 0.332) and RA patients (*P* < .001, r = 0.402).

3.3 | Correlation of baseline miR-125a and miR-125b with clinical characteristics in RA patients

Baseline miR-125a positively correlated with CRP level (*P* < .001, r = 0.395), and baseline miR-125b positively correlated with TJC (*P* = .004, r = 0.292), SJC (*P* = .009, r = 0.266), ESR level (*P* = .001, r = 0.333), CRP level (*P* < .001, r = 0.515), and DAS28-ESR score (*P* < .001, r = 0.414) in RA patients (Table 2).

3.4 | Clinical response rate, miR-125a and miR-125b at different time points in RA patients

After receiving IFX treatment, 28 (29.2%), 49 (51.0%), and 69 (71.9%) RA patients achieved clinical response at W4, W12, and W24, respectively (Figure 2A). During the IFX treatment throughout 24 weeks, miR-125a relative expression at W0, W4, W12, and W24 was 1.978 (1.304-3.419), 1.669 (1.006-3.013), 1.316 (0.776-2.431), and 1.189 (0.636-2.275), respectively, which exhibited a downward trend (*P* < .001) (Figure 2B). MiR-125b relative expression at W0, W4, W12, and W24 was 2.420 (1.578-3.695), 1.937 (1.095-2.603), 1.612 (0.798-2.338), and 1.417 (0.676-2.220), respectively, which also displayed a decreasing trend (*P* < .001) (Figure 2C).

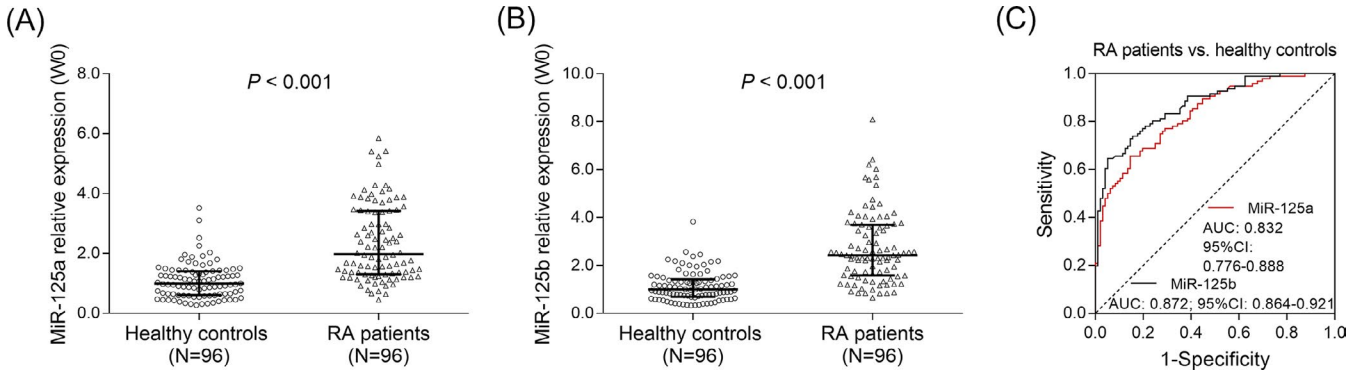

FIGURE 1 MiR-125a and miR-125b in RA patients and healthy controls. Comparisons of miR-125a (A) and miR-125b (B) relative expressions between RA patients and healthy controls. ROC curve analysis of miR-125a and miR-125b in distinguishing RA patients from healthy controls (C). AUC, area under the curve; CI, confidence interval; MiR-125a, microRNA 125a; miR-125b, microRNA 125b; RA, rheumatoid arthritis; ROC, receiver operating characteristic

TABLE 2 Correlation of miR-125a/b with clinical characteristics at baseline

Items	MiR-125a		MiR-125b	
Continuous variables	**Spearman r**	***P* value**	**Spearman r**	***P* value**
Age	−0.061	.556	0.050	.626
BMI	0.190	.064	−0.034	.745
Disease duration	0.062	.552	0.085	.410
TJC	0.145	.158	0.292	.004
SJC	0.167	.104	0.266	.009
ESR	0.146	.154	0.333	.001
CRP	0.395	<.001	0.515	<.001
DAS28-ESR score	0.192	.061	0.414	<.001
HAQ-DI score	0.109	.289	0.132	.200
Items	**MiR-125a**		**MiR-125b**	
Categorical variables	**Median (IQR)**	***P* value**	**Median (IQR)**	***P* value**
Gender, No. (%)		.672		.208
Female	2.224 (1.438-3.371)		2.267 (1.751-2.547)	
Male	1.953 (1.290-3.433)		2.532 (1.544-3.779)	
History of biologics, No. (%)		.493		.181
No	2.117 (1.281-3.449)		2.484 (1.709-3.751)	
Yes	1.701 (1.405-2.569)		2.237 (1.482-2.671)	
RF status, No. (%)		.250		.698
Negative	2.521 (1.243-4.077)		2.337 (1.298-4.174)	
Positive	1.878 (1.275-2.934)		2.547 (1.583-3.684)	
ACPA status, No. (%)		.128		.661
Negative	2.231 (1.547-3.886)		1.786 (1.244-2.975)	
Positive	2.387 (1.367-4.343)		2.547 (1.559-3.744)	
Combined medications, No. (%)		.339		.105
MTX	2.107 (1.405-3.446)		2.685 (1.633-3.953)	
LEF	1.918 (1.210-3.780)		2.306 (1.480-2.291)	

Note: Correlation was determined by Spearman's rank correlation test or Wilcoxon rank-sum test.

Abbreviations: ACPA, anti-citrullinated protein antibodies; BMI, body mass index; CRP, C-reactive protein; DAS28-ESR score, disease activity score in 28-joint count-erythrocyte sedimentation rate; ESR, erythrocyte sedimentation rate; HAQ-DI, health assessment questionnaire disability index; IQR, interquartile range; LEF, leflunomide; MTX, methotrexate; RF, rheumatoid factor; SJC, swollen joint count; TJC, tender joint count.

3.5 | Correlation of baseline miR-125a and miR-125b with clinical response to IFX at W24 in RA patients

RA patients were divided into responders (n = 69; 71.9%) and non-responders (n = 27; 28.1%) based on clinical response status at W24. Baseline miR-125a (*P* = .009) (Figure 3A) and miR-125b (*P* = .002) (Figure 3B) relative expressions were higher in responders than that in non-responders.

3.6 | Analysis of factors predicting W24 clinical response to IFX in RA patients

Univariate regression analysis revealed that higher miR-125a (*P* = .010, OR = 1.809), higher miR-125b (*P* = .003, OR = 2.036), and higher CRP (*P* = .005, OR = 1.034) correlated with better W24 clinical response, while higher disease duration (*P* = .031, OR = 0.871) and history of biologics (*P* = .006, OR = 0.223) correlated with worse W24 clinical response in RA patients (Table 3). Further forward stepwise multivariate logistic regressions analysis disclosed that higher miR-125b (*P* = .041, OR = 1.853) and higher CRP (*P* = .023, OR = 1.033) were independent predictive factors for better W24 clinical response, while higher disease duration (*P* = .003, OR = 0.758) and history of biologics (*P* = .026, OR = 0.235) were independent predictive factors for worse W24 clinical response in RA patients.

3.7 | ROC curve analysis

Independent predictive factors for W24 clinical response to IFX in RA patients were used to construct the predictive model by

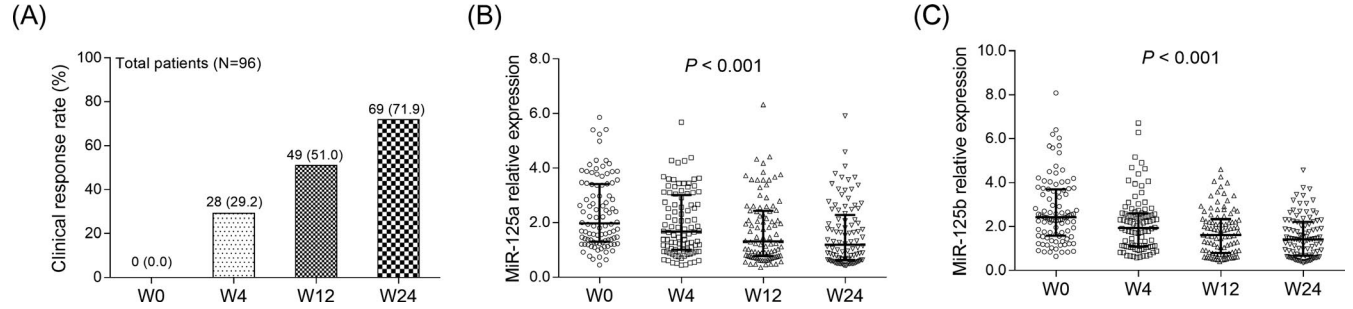

FIGURE 2 Clinical response rate, miR-125a and miR-125b at W0/W4/W12/W24. Clinical response rate to IFX in RA patients at W0/W4/W12/W24 (A). MiR-125a (B) and miR-125b (C) relative expressions at W0/W4/W12/W24 in RA patients. IFX, infliximab; miR-125a, microRNA 125a; miR-125b, microRNA 125b; RA, rheumatoid arthritis; W, Week

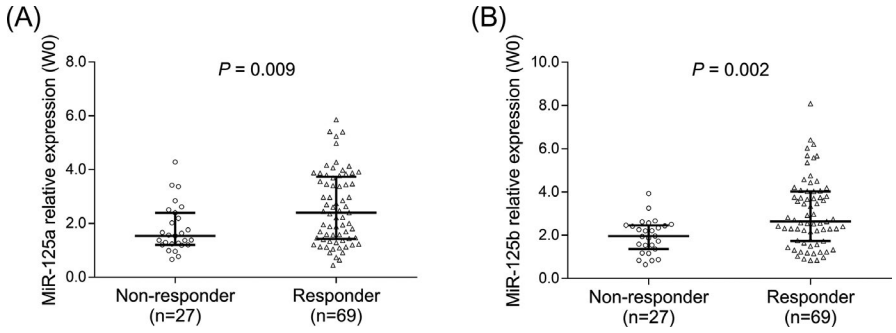

FIGURE 3 Baseline miR-125a and miR-125b in responders and non-responders. Comparisons of baseline miR-125a (A) and miR-125b (B) relative expressions between responders and non-responders. MiR-125a, microRNA 125a; miR-125b, microRNA 125b

forward stepwise multivariate logistic regression with the formula as follows: P = e^ (0.147 + 0.617 miR-125b-0.277 disease duration-1.447 history of biologics + 0.033 CRP)/(1 + e^ (0.147 + 0.617 miR-125b-0.277 disease duration-1.447 history of biologics + 0.033 CRP)), −2Ln(L) = 101.500. Then, the performance of the predictive model and each independent predictive factor were further analyzed by ROC curve analysis. It was showed that miR-125b (AUC: 0.707, 95% CI: 0.604-0.811), disease duration (AUC: 0.676, 95% CI: 0.563-0.789), and CRP (AUC: 0.738, 95% CI: 0.626-0.849) could predict W24 clinical response, while history of biologics (AUC: 0.627, 95% CI: 0.495-0.759) could predict W24 clinical response to some extent (Figure 4). As for the predictive model, it presented a good predictive value for W24 clinical response (AUC: 0.826, 95% CI: 0.788-0.936).

4 | DISCUSSION

Although the pathogenesis of RA is still obscure, a growing body of evidence reveals that epigenetic mechanisms, the most widely studied which is the miRNAs, contribute to the development and progression of RA.[1,12] Among these identified miRNAs, miR-125a and miR-125b play a vital role in modulating inflammatory pathway signaling, which have been reported with the potential as biomarkers for assessing disease activity or predicting clinical outcome in inflammatory diseases including RA.[7-9] For instance, RA patients exhibit higher plasma expression of miR-125a compared with healthy subjects, and miR-125a discriminates RA patients from healthy subjects.[7] Regarding miR-125b, it is raised in RA serum, synovial tissues, and lipopolysaccharide-stimulated fibroblast-like synoviocytes, which enhances the inflammation by activating NF-κB pathway to upregulate TNF-α, IL-6, and IL-1β expressions in RA.[9] Clinically, miR-125b is inclined in both serum and synovial tissues of RA patients compared with healthy controls and trauma amputation patients.[9] Meanwhile, elevated serum expression of miR-125b exhibits the potential as a predictive biomarker for good clinical response to rituximab three months later in RA patients.[8] In the light of the above evidence, we hypothesized that miR-125a and miR-125b might have the implications as biomarkers for discriminating RA patients from healthy volunteers and predicting clinical response to IFX in RA patients. However, no related studies have published yet.

In the present study, we detected the miR-125a and miR-125b expressions in 96 active RA patients who about to receive IFX treatment and 96 healthy controls. It was observed that miR-125a and miR-125b were higher in RA patients compared with healthy controls, and they had good values in discriminating RA patients from healthy controls. Besides, the present study also disclosed that miR-125a correlated with higher CRP level, and miR-125b correlated with elevated TJC, SJC, ESR, CRP, and DAS28-ESR score in RA patients. Herein, the possible explanations were as follows: (a) MiR-125a and miR-125b might induce the release of chemokines and cytokines such as TNF-α and IL-6 via its downstream pathways such as NF-κB signaling pathway, which exaggerated the inflammatory responses in RA.[9,12] Hence, miR-125a and miR-125-b could distinguish RA patients from healthy controls, and they also correlated with worsen disease activities in RA patients. (b) MiR-125b might enforce inflammatory activation of macrophages, elevate co-stimulatory factor expression, and increase responsiveness to IFN-γ, which subsequently potentiated inflammatory responses in RA.[13] Hence, miR-125b could

TABLE 3 Factors predicting clinical response at W24

	Logistic regression model			
			95% CI	
Items	*P* value	OR	Lower	Higher
Univariate regression				
Higher miR-125a	.010	1.809	1.152	2.842
Higher miR-125b	.003	2.036	1.282	3.233
Higher age	.105	0.963	0.921	1.008
Male	.192	0.414	0.110	1.556
Higher BMI	.193	1.110	0.949	1.299
Higher disease duration	.031	0.871	0.768	0.987
History of biologics	.006	0.223	0.076	0.653
RF positive	.729	1.214	0.405	3.637
ACPA positive	.153	2.190	0.747	6.426
Higher TJC	.066	1.170	0.990	1.382
Higher SJC	.150	1.113	0.962	1.286
Higher ESR	.618	1.005	0.986	1.023
Higher CRP	.005	1.034	1.010	1.058
Higher DAS28-ESR score	.153	1.617	0.837	3.126
Higher HAQ-DI score	.609	1.558	0.284	8.537
Combined medications (MTX vs. LEF)	.670	0.824	0.337	2.013
Forward stepwise multivariate logistic regression				
Higher miR-125b	.041	1.853	1.025	3.348
Higher disease duration	.003	0.758	0.632	0.910
History of biologics	.026	0.235	0.066	0.843
Higher CRP	.023	1.033	1.004	1.063

Note: Factors predicting W24 clinical response were analyzed by univariate logistic regression, and the factors with *P* value <.05 in univariate logistic regression were further analyzed in forward stepwise multivariate logistic regression. The forward stepwise multivariate logistic regression model was as follows: *P* = e^ (0.147 + 0.617 miR-125b-0.277 disease duration-1.447 history of biologics + 0.033 CRP)/(1 + e^ (0.147 + 0.617 miR-125b-0.277 disease duration-1.447 history of biologics + 0.033 CRP)), −2Ln(L) = 101.500.

Abbreviations: ACPA, anti-citrullinated protein antibodies; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; DAS28-ESR score, disease activity score in 28-joint count-erythrocyte sedimentation rate; ESR, erythrocyte sedimentation rate; HAQ-DI, health assessment questionnaire disability index; LEF, leflunomide; MTX, methotrexate; OR, odds rate; RF, rheumatoid factor; SJC, swollen joint count; TJC, tender joint count.

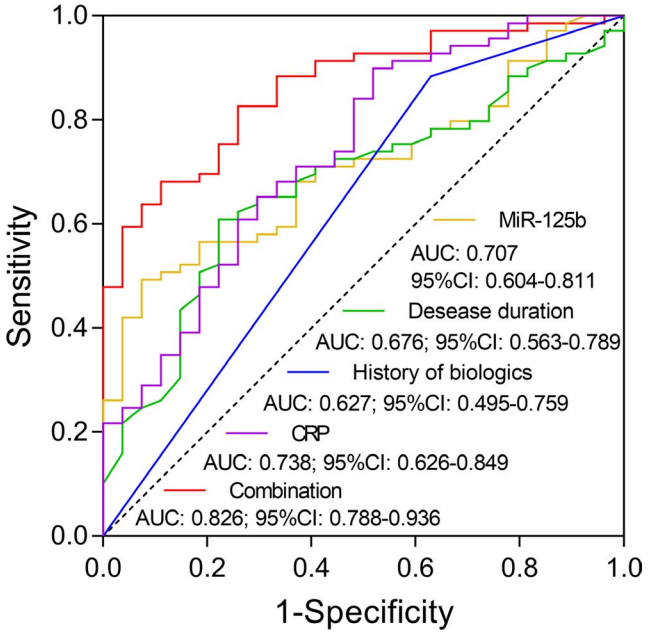

FIGURE 4 The value of the predictive model and each independent predictive factor. The performance of independent predictive factors (miR-125b, disease duration, history of biologics, CRP) and the predictive model (combination of independent predictive factors) in predicting W24 clinical response to IFX in RA patients. AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; IFX, infliximab; miR-125b, microRNA 125b; RA, rheumatoid arthritis; W24, week 24

differentiate RA patients from healthy controls, and it was associated with aggravated disease activities in RA patients.

In the present study, it was displayed that the clinical response rate was increased gradually with the treatment of IFX, while miR-125a and miR-125b were decreased gradually with the treatment of IFX during the 24-week follow-up period. These could be explained by that: (a) MiR-125a and miR-125b might mediate their downstream pathways (eg, NF-κB signaling pathway) to upregulate cytokines and chemokines (eg, IL-β, IL-6, TNF-α), which contributed to the excessive inflammatory responses in RA.[9,12] As clinical response to anti-inflammatory treatment (IFX) was increased, the inflammation was decreased. Hence, miR-125a and miR-125b expressions were reduced gradually with IFX treatment. (b) Based on our previous finding, miR-125a was and miR-125b was associated with elevated inflammation (higher CRP). With the IFX treatment, clinical response rate was increased gradually, and inflammation was reduced. Hence, miR-125a and miR-125b were decreased gradually with IFX treatment in RA patients.

Additionally, the present study revealed that baseline miR-125a and miR-125b were highly expressed in responders, and they both could predict better W24 clinical response to IFX in RA patients. While only miR-125b could independently predict W24 clinical response to IFX. Herein, we proposed several possible explanations: (a) Based on our previous finding, higher miR-125a and miR-125b expressions correlated with higher inflammation (higher CRP level). CRP levels are considered as an indirect marker for TNF production since production of TNF results in elevated CRP level.[14,15] RA patients with higher CRP levels might benefit more from anti-TNF treatment than these with lower CRP levels. Thereby, miR-125a and miR-125b could predict better clinical response to IFX in RA patients. (b) Based on our previous finding, higher miR-125b expression was associated with amplified inflammation (higher CRP level) and exacerbated disease activity at baseline (higher DAS28-ESR score), which resulted in a larger reduction gap of DAS28-ESR score and a better

clinical response to IFX. Thereby, higher baseline miR-125b was associated with better clinical response to IFX in RA patients. (c) MiR-125b was an independent predictive factor for clinical response to IFX in RA patients, which might be explained that miR-125b affected the TNF inhibitor sensitivity through regulating several critical genes and pathways, and subsequently led to a better clinical response to IFX. However, this hypothesis needed further validation.

However, several limitations should be considered. Firstly, the cases in the present study were all previous DMARDs-treated RA patients with a relative long disease duration, thereby the predictive value of miR-125a and miR-125b for clinical response to IFX in treatment-naïve patients needed to be further explored. Secondly, as the present study is a non-interventional study, some confounding factors (eg, history of biologics and combination medication) might affect the evaluation about the predictive value of miR-125a and miR-125b for clinical response to IFX in RA patients; therefore, we performed forward stepwise multivariate logistic regression analysis to reduce the effect of confounding factors. Thirdly, the exploration of detailed mechanism regarding miR-125a and miR-125b in clinical response to IFX in RA patients should be warranted in future. Lastly, clinical response to IFX was defined within a relatively short duration of treatment (24 weeks), thereby the predictive value of miR-125a and miR-125b for long-term clinical response to IFX in RA patients should be investigated in future.

To conclude, monitoring of circulating miR-125a and miR-125b might provide insights into the optimization of personalized treatment strategy and the prognosis improvement of RA patients in clinical practice. Further validation studies and preclinical assessment of underlying molecular mechanism are greatly needed to verify our findings.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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